

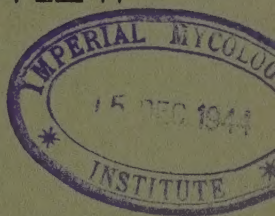
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THE BOTANICAL REVIEW

Interpreting Botanical Progress



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THE BOTANICAL REVIEW

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No. 7

METABOLIC PHENOMENA ASSOCIATED WITH VIRUS INFECTION IN PLANTS

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University of Illinois

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INTRODUCTION

Significance of Physiological Changes

An almost unbelievable number of papers has been published during the last 50 or 60 years bearing on the general subject of plant diseases caused by filterable viruses. Several papers have mentioned the desirability of a knowledge of the physiological disturbances which accompany this type of disease because such knowledge might contribute to a better understanding of the nature and cause of the diseases themselves. Whitehead (137), as late as 1931, stated that comparatively little attention had been directed to the metabolism of virus-infected plants, and yet, since the original publication of Woods (139) in 1899, there had been published 138 articles containing data bearing on this phase of pathological physiology. The purpose of the present paper is to review this mass of widely scattered data and to present as nearly as possible a comprehensive picture of what happens to the physiological processes of plants when they are disturbed by virus infection.

The viewpoint now generally held is that virus diseases are initiated by filterable, toxic substances which are usually highly infectious. The living or non-living nature of virus does not fall within the limits of the present review. Consideration of the symptoms of infection, unless clearly referable to some specific physiological behavior, is also beyond the concern of the present paper.

Virus Movement Within the Plant

In a later section, evidence is presented which indicates that the course of certain physiological disturbances is related to the spread of the virus through the plant. Holmes (63) inoculated various areas of the leaves of *Nicotiana tabacum* with mosaic virus and determined the time necessary for an infectious concentration of the virus to appear in other parts of the plant. New virus developed in high concentration near the site of the original inoculation, and visible lesions were present after four days. The systemic or secondary phase appeared at about the eighth day and was marked by the simultaneous appearance of the virus in the petiole of the inoculated leaf, in all parts of the stem, in the young leaves at the tip, and in the root. After this, the virus appeared progressively in the leaves downward from the tip, and upward from the root. All parts of the plant became infectious at about the fourteenth day. Since the presence of the virus was determined by inoculating the press juice into healthy plants, it is obvious that the virus actually had reached the studied parts sometime previous to the time when its detectable infectious concentration was observed.

The actual time of arrival of the virus in various parts of the plant was studied in greater detail by Samuel (111) who refined the technique of its detection. Various parts of the tomato plant were stored for one week in test tubes which permitted very small, non-infectious amounts of virus to increase to an infectious concentration. The press juice from this aged tissue was inoculated into healthy plants in the usual manner. Virus did not move from an inoculated leaf for at least three or four days. When virus leaves the leaf, it spreads through the vascular system with great speed, and first reaches the roots. So rapid is this distribution that intervening stages are scarcely detectable. About one day later, the virus reaches the tip of the plant, the intervening stages being accomplished also with considerable speed. In young plants, complete invasion of all parts of the plant soon follows, but in medium-sized plants, about three weeks pass before complete invasion occurs, and in mature fruiting plants, two months are necessary. Mature leaves on large plants remain free from the virus for three months or more except for a limited movement along their midribs.

Extensive reviews of the problem of the movement of virus through the plant from the point of infection have been published.

From the very considerable literature it appears that the virus spreads slowly from cell to cell from the point of infection by means of the plasmodesma. After three to five days the phloem is invaded, and very quickly thereafter the material appears in the roots; this is followed, in a matter of hours, by its appearance in the top of the plant by way of the phloem. The period during which distribution of the virus through the phloem is accomplished coincides, in general, with the period of maximum physiological disturbance of the metabolism of the mosaic-infected tobacco plant (143, 144, 145).

Phloem Necrosis

Changes in the vascular system of virus-infected plants have a direct bearing on the problems of accumulation and distribution of chemical substances during the course of the disease. This is particularly true in respect to the phloem.

The view that phloem necrosis follows soon after virus infection was first presented by Quanjer. This author's initial publication (95) on this problem presented a series of drawings showing changes in the vascular system of virus-infected potato plants. Disintegration of phloem was held to be the cause of the symptoms of leaf-roll. This opinion was immediately attacked (113) by pointing out that the potato plant is unusually sensitive to phloem disturbances and that such factors as high rates of transpiration without adequate water supply, insufficient supply of inorganic nutrients and infection by *Phytophthora* would all cause phloem necrosis and consequently a rolling of the leaves. Consequently, phloem necrosis was regarded as the result of the disease and not the cause.

Disintegration of phloem also was found to be associated with infections by *Bacillus dianthi* (118, 119), although the authors believe that this soil-inhabiting saprophyte was a secondary invader and not necessarily the cause of curly-top of sugar beets. This *Bacillus* entered the plant independently of the insect *Eutettix tenellus* which infected the sugar beet with the virus.

Quanjer (96) continued his study despite doubts cast on its significance and soon published a detailed description of changes associated with disintegration of the phloem. In potato plants, the cell walls became swollen, pressed together, and later their cavities were obliterated. Necrosis was most marked in the oldest phloem strands near groups of bast fibers. It appeared first at the tip of the plant, and gradually extended downward from the mid-

ribs of the upper leaves, finally through the underground portion of the stem to near the tuber. The smaller leaf-veins, the new tubers and roots were not affected. An unusually fine series of plates and further details (2) verified that phloem necrosis of potato showing leaf-roll symptoms was more pronounced in the tip region. The primary phloem was the first to suffer. At first, the cell walls separated in a localized group of phloem cells which formed intercellular spaces filled with a yellow substance. Later, the primary walls of cells bordering these cavities also became discolored. The spaces finally became so extensive that the phloem collapsed into a disorganized mass. The phloem cells themselves frequently showed accumulations of granular or globular deposits which were probably remnants of protoplasm. The diseased walls gave a positive test for cutin but not for lignin. The numerous distinctly isolated groups of necrotic cells suggested that the causative virus agent was formed *in situ*.

Neger (85) again raised the question as to whether phloem necrosis was the cause or an effect of the disease. He assumed the view that death of the phloem cells was brought about by undernourishment caused by prevention of the proper rise of sap, although he did not attempt an explanation of why the sap did not rise.

The extent of phloem necrosis has been used (98) as a basis for classifying various types of virus diseases of potatoes. The terms, potato "curl", "leaf-roll", "curly-dwarf" and "mosaic", have been variously applied to the disease, but Quanjer pointed out that "leaf-roll" should be called "phloem necrosis" and that "leaf-curl" or "curly-dwarf" should be called "mosaic", since this condition is merely an intense form of phloem necrosis. Mild disturbances in the upper leaves were called "primary leaf-roll". The progeny from plants with primary leaf-roll show a more intense disturbance, and this condition was called "secondary leaf-roll". Tobacco plants also were shown to develop phloem necrosis in association with the mosaic condition.

Phloem necrosis finally came to be a recognized phase of virus diseases for plants generally. It has been discovered in the Malvaceae (77), in the coffee plant (124), in beet (76), and associated with peach-yellows and peach-rosette (117). The Japanese have reported that it accompanied spike disease of sandal, mulberry-dwarf, and stripe disease of sugar cane.

The significance of phloem necrosis in explaining the accumulation of carbohydrates in leaves will be discussed later.

RESPIRATION

Gaseous Exchange

Green Plants. Observations on respiratory rates are important in many diverse types of physiological studies. Their significance does not lie in any specific interpretation of the factors affecting the changes in the amount of oxygen consumed or in the carbon dioxide eliminated, for our knowledge of the chemical processes involved in respiration is not yet complete enough to permit always such specific explanations, but their real significance lies in the fact that almost any change in metabolism is reflected in the amount of energy involved, and this is detectable usually in the respiratory rates. The use of fever thermometers by physicians is of little help in diagnosis, but serves only as an indication that something is wrong with the physiology of the patient.

The first comment that virus-infected plants were suffering from a "fever" was made by Bunzell (27), and a year later he suggested (29) that a study of the respiratory rates of sugar beets infected with curly-top might serve as a basis for a more exact interpretation of the nature of the disease. Such studies were planned by the Office of Plant Physiology and Fermentation Investigation of the United States Department of Agriculture, but the first published data did not appear until fourteen years later when Thung (130), working in Holland, published his study on leaf-roll of potato. Healthy mature leaves eliminated an average of 8.01 milligrams of carbon dioxide per gram of original dry substance per hour, while diseased leaves eliminated 3.38 milligrams. The higher respiration rate of the diseased leaves was evident also when referred to the original fresh weight of the tissue. A significant aspect of Thung's work is that it showed that the delayed or inhibited disappearance of starch from diseased leaves, which will be discussed in detail later, did not depend on its slower utilization as a respiratory substrate. What little starch did disappear from the diseased leaves was adequately accounted for by the loss of carbon as carbon dioxide.

Dunlap (45) studied the rates of carbon dioxide production by virus-infected plants of tobacco, tomato, pokeweed, cucumber,

raspberry, spinach and aster, and found that in all cases young infected leaves had a higher respiration rate. However, mature leaves usually gave less carbon dioxide. This change with age has considerable significance, as will appear later (143). A more detailed study (46) of a larger number of species verified the earlier data.

Whitehead (137), in 1931, lamented the comparatively small amount of attention given to the metabolism of virus-infected plants, although 89 papers already had appeared treating various aspects of physiological disturbances of plants infected with virus. His own very thorough study of leaf-roll of potatoes showed that the tubers eliminated carbon dioxide more rapidly per gram of fresh or dry weight when they were first harvested, but during storage the rate progressively dropped until it was even less than the rate of healthy tubers. On sprouting, this lessened rate was maintained by the tuber, although the sprouts, especially after they had broken into leaf, showed a greatly stimulated respiration. This accelerated rate was noted long before visible rolling of the leaf appeared, although not necessarily before sugars had begun to accumulate. It is interesting to note that the rate of anaerobic elimination of carbon dioxide by the leaves and tubers was not affected by the disease.

The data of Dunlap (45, 46) were criticized (31) on the grounds that Dunlap was measuring the rate of respiration in an atmosphere containing less than the normal amount of oxygen. This criticism seems invalid because of the comparatively low rate of respiration and considerable volume of Dunlap's respiration tubes. It is known, moreover, that the rate of plant respiration does not change significantly with oxygen concentration, except in the lower ranges. The further criticisms that the alkali probably did not absorb the carbon dioxide as rapidly as it was produced and that the duration was too short, also seem to be small points for argument. Caldwell (31) extended the period of observation to 48 hours and showed a progressively decreasing rate which indicates some type of injury, or at least, abnormal circumstance. In view of this fact, the shorter, ten-hour period (46) which he criticized should give the most valid date. But in spite of these possible sources of error, his data (31) agreed essentially with earlier work (45, 46) and showed that virus-infected tomato plants had a higher respiration rate, whether

expressed in terms of the initial fresh weight, dry matter or nitrogen content.

The only author unable to detect an increased respiratory rate (76) studied healthy and mosaic-infected tobacco plants with a refined manometric technique. He found that the diseased leaf tissue had a lower rate of respiration than normal.

Wynd (143) observed the oxygen use of normal and mosaic-inoculated tobacco plants by a highly sensitive monometric procedure at two or three day intervals for 21 days after inoculation, and found that the rate of respiration was greatly increased by the fourth day after a lower leaf was inoculated. This period of disturbed metabolism occurred simultaneously throughout the plant and preceded by about ten days the general appearance of the virus in infectious concentration. By the time the press juice became infectious at about the fourteenth day, the rate of oxygen use was always less than that of normal leaves. It thus appeared that the data of Dunlap and of Lemmon were not necessarily contradictory, since the plants had either an increased or lessened rate, depending on the time which had elapsed since inoculation. This further agrees with the difference between young and old leaves (45, 46), and there is at least a qualitative agreement with the changes in respiration rate of virus-infected potato tubers during storage (137).

Virus Preparations. Many approaches have been made to the problem of the living or non-living nature of virus particles, but in spite of the great number of papers published there yet remains confusion among the interpretations. Consequently, there is considerable interest in the possible respiration of virus preparations, since this would have a bearing on their living or non-living nature.

Bronfenbrenner (21, 22) studied bacteriophage preparations under conditions which permitted detection of as little as 0.02 milligram of carbon dioxide, but he was unable to detect any respiratory activity over periods of several hours. Wohfeil (138) also was unable to detect vital activity by bacteriophage particles. Bachmann and Wohlfeil (4) could detect neither carbon dioxide elimination nor oxygen absorption by bacteriophage preparations in the presence of the dead cells of sensitive strains of bacteria. Parker and Smythe (90) could detect neither carbon dioxide elimination nor oxygen use by concentrated preparations of vaccine virus. In order

to assure a suitable substrate for respiratory activity, glucose and glucose monophosphate were added, but negative results were again obtained. An extensive study of the problem was reported by Wynd and Bronfenbrenner (146) who used cultures of *Staphylococcus aureus* lysed by bacteriophage which contained in the order of ten billion active particles of bacteriophage per cubic milliliter. The micro-manometric procedure used was sensitive to 0.0002 milligram of oxygen absorption, and even under these conditions, uniformly negative results were obtained in a long series of experiments.

On the other hand, McKinley and Coulter (83) detected a slight increase in the amount of carbon dioxide produced by a strain of *Escherichia coli* when growth occurred in the presence of active bacteriophage. These authors were probably correct in considering this increase to be due to an increased growth rate of the bacteria rather than to any metabolic activity of the bacteriophage particles themselves.

Eaton (48) also studied cultures of *Staphylococcus aureus* by a manometric procedure and reported an observable uptake of oxygen and also production of carbon dioxide. He pointed out that the oxygen absorption might be due to a purely chemical auto-oxidation of some component in the medium, but that the carbon-dioxide production indicated a living metabolic activity. His suggestion that the medium might absorb oxygen on its own account, is in error, as shown by other data (146).

The living nature of virus particles was assumed without reservation by Pirie and Holmes (92) who reported that preparations of the virus of agalactia, a disease of the mammary glands of goats, reproduced in culture and exhibited a number of phenomena characteristic of living cells.

Oxidizing Enzymes

Oxidases.

a. *Oxygenase*: It is difficult to relate the activities of the oxidizing enzymes to respiratory activity. They are conventionally regarded as respiratory enzymes only because they catalyze oxidation reactions *in vitro*. The author will present a critical review of these assumed relationships in the future, but for the present they will be considered in the conventional sense. Oxygenase, peroxidase

and tyrosinase are grouped arbitrarily in the present discussion under the generic term of oxidases.

The original article (139) concerning oxidizing enzymes in mosaic-infected tobacco reported that the light colored spots of leaves were found to have about twice the oxygenase activity as the greener areas. These observations were again published (141) in a later article. A classic paper (59) appeared in 1900 which substantially verified the data (139) for tobacco mosaic, and extended the observations to peach-rosette and peach-yellows. A similar increase was observed in mosaic-infected tobacco and tomato (34). A more thorough later study (35) showed that both the leaves and roots of mosaic-infected tobacco had increased oxygenase.

Wynd (144) followed the sequence of enzymatic changes in tobacco at two- or three-day intervals for 21 days after a lower leaf had been inoculated with the mosaic virus. There was a diminished activity in the four leaves originally on the plant which reached a minimum on the fourth to the sixth day. After six days another leaf at the tip was large enough to be included in the study. This fifth leaf reached its minimum on the tenth to the twelfth day. The sixth leaf was included in the study on the eleventh day and its minimum was reached on the fourteenth day. All leaves, therefore, seem to go through a preliminary period of lessened oxygenase activity, and the leaves which appear later on the plant show the minimum activity at a progressively later date. All leaves from the inoculated plants show a comparatively abrupt increase with a maximum at about the fourteenth day. Following this, the relative activity diminishes until it is below that of normal leaves. In general, these relationships are more pronounced in the lower leaves of the plant. Purified tobacco mosaic was shown (106) to have no oxygenase activity.

The mulberry-dwarf disease is accompanied by greatly increased oxygenase (127, 128, 129). Sometimes this increase is as much as 500%. Leaf-roll-infected mulberry trees also show a more active oxygenase (116). The cause of this increase was held to be the accumulation of organic foods due to a disturbance in translocation.

The leaves and roots of virus-blighted spinach were tested manometrically (30) for their ability to oxidize a great many oxygenase reagents. Although all reagents did not yield similar results there was a general indication of increased oxygenase activity.

The economic significance of leaf-roll of potatoes has led to considerable effort to detect signs of the disease in the tuber before planting. Unfortunately, the oxidizing enzymes vary greatly in normal tubers, which lessens the value of their measurement as a means of detecting the virus. Very erratic data (40, 41) show possibly a slight increase in oxygenase in resting virus-infected tubers. Leaf-roll of potato is accompanied (110) by a higher rate of oxygenase activity in the leaves, stems, roots and tubers. Seeds showed no difference. Further studies (29) on curly-dwarf of potatoes showed that the diseased plants had an increased activity of oxidizing enzymes in both leaves and roots. These qualitative differences agree generally with the rates of respiration.

Increased oxygenase activity in virus-infected grape leaves has been interpreted (89) to be the cause of an increased respiration rate.

Extensive studies of curly-top of sugar beet (25, 26, 27, 28) have shown that oxidizing enzymes in leaves and roots are always significantly increased by the disease. This increase accompanied inhibited growth, whether caused by excessive drought, excessive watering or other factors.

Hasselbring and Alsberg (58) did not believe that virus diseases were accompanied by increased activity of oxidizing enzymes but rather by decrease in antiooxidase.

The only publication that has come to our notice concerning changes in oxidase activity in animals suffering from a virus disease, reports increased oxidase activity in the lymph of guinea pigs infected with hoof-and-mouth disease. In general, the activity paralleled the virulence of the disease (93).

b. Peroxidase: The earliest observations of the effects of virus infection on the activity of peroxidase (139, 140, 141) showed that this enzyme was about twice as active in light colored areas of mosaic tobacco leaves. These data were verified (59) for tobacco and the same condition existed in peach-yellows and peach-rosette. Mosaic-infected tobacco and tomato also exhibited increased peroxidase activity (34).

The course of changes in peroxidase in tobacco was observed (144) at two- or three-day intervals from the time a lower leaf had been inoculated. In general, there was a preliminary decrease below that of normal plants, but this soon was followed by an in-

crease. The changes in peroxidase were not as well marked as those of oxygenase, and the magnitude of the increase was not as great as reported by other workers. In later stages of the disease, there was a gradual diminution of peroxidase activity until it was less than that of normal leaves.

Crystalline preparations of tobacco mosaic virus were shown (106) to have no peroxidase activity.

Mulberry-dwarf disease is accompanied by greatly increased peroxidase activity (127, 128, 129). In many instances the increase is as great as 500%. This has been attributed (116) to an accumulation of organic food reserves brought about by disturbed translocation, and also to the diminution of an antioxidase (58), rather than to increase of the oxidation enzymes themselves.

Attempts have been made (40, 41) to detect the presence of leaf-roll virus in potato tubers by measuring the activity of peroxidase, but this enzyme varies so greatly in healthy tubers that it has little value as a diagnostic character for the diseased condition. Sometimes there was very great increase and sometimes none at all, but in general there seemed to be augmented activity of this enzyme. This same condition existed in the leaves of curly-dwarf potato plants (29, 86). These observations (110) have been extended to various parts of diseased potato, and increased peroxidase in the leaves, stems, roots and tubers was always found. Seeds from diseased plants did not show any difference in this enzyme.

Curly-top of sugar beets (25, 26, 27, 28) called forth great increase in oxidizing enzymes, but this was not necessarily limited to virus infection. Any factor inhibiting normal growth, such as nutritional disturbances, abnormal water relations, or other diseases was equally effective.

Virus-infected grape leaves (89) exhibited greater peroxidase activity, which has been interpreted to be a factor causing the stimulated carbon-dioxide elimination.

Very few data have been published concerning the effect of virus infection of animals on the enzymatic activity of their tissue. The peroxidase activity (36) of milk from cows suffering with hoof-and-mouth disease remained unchanged. On the other hand, oxidase activity of the lymph of guinea pigs increased with the virulence of the infection with this disease (93).

c. Tyrosinase: Observations on tyrosinase have been made (40) in an effort to detect virus-infected potato tubers. Unlike oxy-

genase and peroxidase, this enzyme was remarkably similar in activity in different healthy tubers, but the effect of virus infection was too small to be used as a diagnostic character for the presence of the disease. In a later study (41) tyrosinase was found to be a little more active in the diseased resting tubers. Sprouting tubers reacted variously, sometimes even exhibiting no tyrosinase activity at all.

The ability of virus-blighted spinach to oxidize a great many different substrates has been studied (30). Healthy leaves were unable to oxidize tyrosine, but blighted leaves gave a detectable reaction. Normal roots gave a definite tyrosinase reaction and this was considerably increased by infection.

Dehydrogenase. There have been no important studies on changes in dehydrogenase activity of plants infected with virus. This is all the more strange in view of the great importance that many authors have placed on changes in respiration.

The difficulty of measuring dehydrogenase in virus-infected potato tubers by observing decolorization of methylene blue was shown (110) to be due to the presence of interfering substances. Although the Chamberlain filter removes these substances, little work has been done because of the difficulty of the procedures.

A few observations have been made on changes in dehydrogenase in animal tissues affected by virus. Increased dehydrogenase activity has been noted (135) in the milk of cows infected with hoof-and-mouth disease, although these data have not been verified (36).

Several authors have attempted to demonstrate dehydrogenase activity in preparations of the virus itself. Agalactia virus cultures were able to reduce methylene blue and to absorb large amounts of oxygen in the presence of lactate (92). Purified tobacco mosaic was unable to cause reduction of methylene blue (106). Others (51) could not detect dehydrogenase activity in preparations of bacteriophage.

Catalase. Suzuki (127, 128) reported that mulberry-dwarf disease called forth increased catalase activity in the leaves. He believed that this increase was brought about by excessive production of hydrogen peroxide as a by-product of stimulated respiration. Chapman (34) suggested that the direct cause of the mosaic diseases of tobacco and tomato was free hydrogen peroxide which resulted from the heightened respiratory activity, yet he found that

catalase was less in the diseased leaves. A more thorough investigation (35) pointed out that dark green tobacco leaves had greater catalase reaction than light green leaves, even though both were healthy. This was true for various leaves on the same plant as well as those from different plants. The dark areas of mosaic leaves contained more catalase than did the light areas. In general, when the leaves were studied as a whole, the catalase decreased to about 50% of normal.

Wynd (144) reported changes in catalase of individual tobacco leaves at two- or three-day intervals from the time that a lower leaf had been inoculated with the mosaic virus. By the sixth day the activity of inoculated plants exceeded that of healthy plants. The activity drops, however, after 11 to 14 days. A small subsequent rise appears after about 18 days. The period of minimum activity of each leaf agreed generally with attainment of the power of its press juice to infect new plants.

Respecting catalase of various parts of virus-infected potato, the variety Wohltmann gave increased activity in the press juice from the top of virus-infected stems, while the variety Klara reacted oppositely (13). Middle and lower portions of the stem showed augmented catalase activity. The author believed that the stimulated catalase was induced by the higher pH of the sap. A study (57) of the relationship between acidity of the juice of tobacco leaves and catalase activity shows convincingly that increased acidity is accompanied by a greater catalase activity, since mosaic-infected tissue contained more acid and less catalase. Curly-top-infected sugar beets showed no difference in their catalase activity (18).

The effect of virus infection on the catalase of animal tissues has been observed by several students. Catalase in milk from cattle with hoof-and-mouth disease increased progressively as the disease advanced (36). This was later verified (135). The catalase in the blood of Guinea pigs infected with the disease was unchanged (93).

Catalase activity was not found in purified tobacco mosaic virus (106), and negative results were also obtained with the bacteriophage of *Escherichia coli* (115). Other workers (51) decided that bacteriophage was non-living because they could find no catalase reaction in lysed bacterial cultures. These data are probably incorrect, since lysed cultures of *Staphylococcus aureus* exhibited a

definite catalase reaction when the method of detection was sufficiently sensitive (145). The activity of the catalase was proportional to the number of cells that had undergone lysis (78, 145). This indicated that the bacteriophage particles themselves were not the agents of the catalysis because they were shown by titration to be present in concentrations of the same order of magnitude in all instances (145).

CARBOHYDRATE METABOLISM

Chloroplast Pigments

Chlorophyll.

a. Amount of chlorophyll: General chlorosis or a mottled appearance of leaves is frequently a symptom of virus infection. This is so obvious that but few quantitative data have been published. Light green areas of mosaic tobacco leaves contain fewer chloroplastids and less chlorophyll per plastid (39), and the pale appearance due to lessened chlorophyll was also observed in tomato, potato, petunia, pepper, *Hyoscyamus*, sweet pea, Canada field pea, kidney bean, Windsor bean and clover. The dark areas of mosaic tobacco leaves contained 30% more chlorophyll *a* and 69% more chlorophyll *b* than did normal green tissue. The light colored areas showed a 46% reduction in the amount of both chlorophyll *a* and *b* as compared to normal leaves. When the entire leaf of mosaic tomato plants was included in the determination there was only 55% as much chlorophyll *a* and about 70% as much chlorophyll *b* as in normal leaves (49).

Quantitative studies (44) of chlorophyll in mosaic tobacco plants at various periods from the seedling to the mature stage show chlorophyll was always less in diseased plants; the diminution was less in young plants, although examination of the author's data shows the effect of age to be of comparatively little importance. In three types of tobacco mosaic, one caused a mild dark green color, one a light green and one an intense yellow. All three types of virus lessened the chlorophyll significantly (91).

The usual extraction procedures used in quantitative determinations of chlorophyll were less efficient with mosaic-infected leaves of *Vicia faba* L. (16), and this exaggerates somewhat the observed differences. This is such a significant point of technique that the same chlorophyll content in both green and light areas of the leaves of sugar beets infected with curly-top has been obtained when

proper precautions have been taken (18). There seems to be no explanation why the dark areas should resist the action of chlorophyll solvents. The greater chlorophyll content in green areas of mosaic sugar beet leaves was held (114) to be partly responsible for their increased carbohydrate content.

b. Cause of diminished chlorophyll: The importance of oxidizing enzymes in mosaic tobacco plants in causing destruction of chlorophyll was first suggested by Heintzel (59) and accepted by Paulsen (see Pantanelli, 89) as applicable also to grape leaves. It has been pointed out (39) that part of the diminution of the total chlorophyll was due not only to the decrease per chloroplastid, but also to the lessened number of chloroplastids. Others (14) believed that the increased acidity which accompanies virus diseases is a factor in the destruction of chlorophyll.

c. Chlorophyllase: Observed changes in chlorophyll content of virus-infected plants suggest that chlorophyllase also is involved. However, a remarkably small amount of attention has been given to this phase of the problem. Peterson (91) found that the yellow color of mosaic-infected tobacco was accompanied by greatly increased activity of chlorophyllase. It is significant that the yellowing and loss of chlorophyll induced by age or nutritional disturbances was accompanied by less chlorophyllase. In normal leaves, high concentrations of this enzyme were correlated with large amounts of chlorophyll, but the reverse obtained for mosaic-infected leaves. Purified tobacco mosaic exhibited no chlorophyllase activity (106).

Carotene. The amount of carotene in mosaic tomato leaves increased 95% over that in normal leaves (49). When only the dark green areas of mottled leaves of tobacco plants were analyzed, they contained 137% more carotene than normal tissue. The light green areas showed an increase of 96%.

Xanthophyll. The only data (49) on the effect of virus diseases on xanthophyll content show that this substance was diminished 64% of normal. Dark areas of mosaic tobacco leaves showed a diminution to 79% and light colored areas to 68% of normal.

Concentration of Carbohydrates

Total Carbohydrates. Carbohydrate metabolism of virus-infected plants has received a great amount of study, and this phase

of the disturbed physiology is probably one of the best known aspects of this group of diseases. Several authors speak of carbohydrates with no mention of the specific types. It is obvious in many instances that starch is the substance referred to. In other papers, the true total carbohydrate was determined. In order to avoid errors in interpreting the meaning of several authors, papers referring to "carbohydrate" will be considered below.

Carbohydrates (127) accumulated to an abnormal extent in mulberry leaves from trees afflicted with mulberry-dwarf. These observations were verified (116).

The behavior of the potato plant shows that carbohydrate accumulation is not a necessary feature of virus infection. Four varieties of potato tubers from seven localities showed that leaf-roll usually accompanied a smaller amount of total carbohydrate when calculated on the fresh weight basis (42). Since diseased tubers had significantly less per cent dry weight, the diminution of carbohydrate is less if calculated on the dry weight basis. The same general relationships were observed during germination and growth in second generation of tubers from diseased stock. Leaves of diseased plants contained less carbohydrate than normal in two varieties and more in one.

The entire aerial parts of potato plants have been analyzed (32) at the stage just before tubers were formed. There was always a higher per cent of total carbohydrate than in normal leaves. Whitehead (137) observed that leaf-roll of potatoes caused accumulation of carbohydrates in the leaves at the expense of the tubers.

Blighted spinach was shown (70) to contain more than a normal amount of carbohydrate.

There was less carbohydrate in the leaves of virus-infected tobacco, tomato and petunia. The author stated that the leaves studied were of the same approximate age as those used for the control. One could assume from this that he (5) believed the relative age to be important, although he did not state what the actual ages were.

Leaves of tobacco, tomato, pokeweed (*Phytolana decandra*), cucumber, raspberry, spinach, and aster infected with yellows had lessened total carbohydrate (45). In a later paper (46) a similar condition was again reported for the above species and the list of plants extended to include peach, plum, and ragweed showing

symptoms of yellows. A more detailed study (47) of carbohydrate metabolism in mosaic tobacco plants again showed that total carbohydrate decreased in the infected plants.

Increased chlorophyll in abnormally dark areas of mosaic sugar beet leaves was believed (114) to be responsible for the augmented carbohydrate content of these areas.

Others (20) found that the total carbohydrates in tomato plants were lessened by the mosaic disease. Since the total nitrogen did not change to a great degree, this resulted in a lower C:N ratio. Polysaccharides were mostly affected. Tomato plants of the varieties Earliana and San Jose Canner, on the other hand, were shown (109) to undergo progressive increase in carbohydrates as the western yellow-blight progressed.

The above data indicate that no definite conclusion can be reached at present concerning the general behavior of the total carbohydrate fraction of plants to virus infection.

Hemicellulose. Hemicelluloses were studied in virus-infected grape (89). In early morning, diseased but yet green leaves contained 98% as much as normal leaves. At the middle of the day the value was 95%, and by evening it had changed to 97%. In leaves in which the disease had progressed to the visible mosaic condition, these percentages were slightly smaller.

Starch and Other Insoluble Carbohydrates.

a. Amount of starch: Carbohydrate fractions designated in the literature as polysaccharides or as insoluble carbohydrates are here assumed to be sufficiently equivalent to starch as to be included in the present discussion. From the earliest period of general interest in the physiology of plant diseases caused by virus, authors have been reporting various types of studies which directly or indirectly involved measurement of the starch content of diseased tissue. Woods (140) observed that even though chloroplastids appeared normal in diseased areas of mosaic tobacco leaves, the cells were visibly gorged with starch. The dark areas of mosaic tobacco leaves were reported (53) to have a higher starch content than the light colored areas, contrary to earlier findings (140). This was verified (39) by using the comparative intensity of iodine staining as the test. Further studies (112) again showed that mosaic-infected leaves of tobacco contained an abnormal starch content, especially in certain areas.

Dunlap (47) extended his previous study of the changes in total carbohydrates of mosaic tobacco leaves to include various fractions. The plants were inoculated with virus while in the seedling stage. In the morning, before synthesis had begun, the ratio of the starch in mosaic to that in normal leaves was 0.40. In the afternoon this ratio increased to 0.57. When the plants were kept in the dark two or three days so that additional synthesis could not take place, the ratio dropped to 0.26, which indicates more rapid disappearance of the starch from the mosaic leaves. When the plants were kept in the cold for two or three days, the ratio dropped to the surprising value of 0.12.

When the leaves of Turkish tobacco were pricked with a pin dipped in a preparation of mosaic virus (64), iodine staining showed that the local lesions at the points of infection were high in starch. This was detectable even before symptoms of the lesions were visible. So definite was this iodine staining test that it permitted identification of virus-invaded tissue of old inoculated leaves which failed to develop visible symptoms. A later study (65), using the same technique, showed that this accumulated starch was not diminished even when the plants were stored in the dark.

Quantitative data were first reported in an extensive study of the physiology of virus infection of grape leaves (89). Observations were made at various times of day and indicated that there was always a significant lessening of starch. This decrease was greater at the middle of the day. Diseased, but yet green, leaves were not as much affected as those which showed visible mosaic symptoms.

Leaves of *Syringa* often attain almost double the weight of equal areas of normal leaves (74), and rough staining tests with iodine show that this increase is due to high starch content accompanying virus infection.

The starch content of the leaves of sandal affected by spike disease is almost double that of normal leaves (37).

Leaves of potato were found (62) to accumulate an abnormal amount of starch as a result of leaf-roll. This accumulation was so great that the authors thought it might mechanically cause the actual rolling of the leaves. These data were later verified (85, 50, 61).

In a study (97) of the starch content of healthy and leaf-roll-infected potato leaves in the early morning before photosynthesis had

begun, the healthy leaves lost much of their starch during the night, while the diseased leaves retained most of their starch synthesized the previous day. This accumulation of starch in the leaves was at the expense of the tubers which significantly decreased their growth as the season progressed. Quanjer (98) later classified the various types of potato leaf-roll according to severity of the symptoms. Healthy leaves contained no starch in the morning, while secondary leaf-roll symptoms were accompanied by a very great amount. Primary leaf-roll symptoms affect only the upper part of the plant with consequent diminution of the degree and extent of starch accumulation. He found that tobacco mosaic virus also was associated with increased starch content.

The most thorough study of starch accumulation (84) used potato plants infected with leaf-roll. Leaflets from diseased plants showed no difference in their starch content for the first five or six weeks of the life of the sprout, but later, starch began to accumulate, even though rolling of the leaves was not yet apparent. The mesophyll of the lower leaves was the first tissue to accumulate starch, even though phloem necrosis began later in the tip of the plant. This remarkably fine article will be referred to later with reference to the cause of carbohydrate accumulation. The increased starch content was confirmed by other workers (32, 130) and found to be as much as 200% or 300% that of normal leaves.

Spinach leaves, blighted with virus infection, have about 80% to 90% more starch than normal leaves (134).

Sugar beet mosaic also was shown (107) to be associated with starch accumulation in the leaves. The author gave no quantitative data but relied on the relative intensity of qualitative tests which apparently were sufficiently sensitive to indicate that great differences existed. The curly-top disease of sugar beets also causes an increased accumulation of starch in the leaves (33), although it has been pointed out that (18) there is little or no starch in the lighter colored areas, according to the severity of the disease.

Western yellow-blight of tomatoes called forth a progressive increase of starch in all parts of the plant (108). When the tomato plant was artificially inoculated with a preparation of mosaic virus (14), the local lesions were soon surrounded by a transient zone of rapid starch translocation. At a later period, localized areas became yellow in color and depleted in starch. Some weeks after in-

fection, starch accumulates in some areas of the leaves and disappears almost entirely from others.

Studies based on the comparative intensity of the iodine staining showed that the lighter colored areas of mosaic leaves had little or no starch, while the dark green areas accumulated this substance to a considerable extent (16). This was true for the virus disease of field pea and clover, and Böning thought it probable that it prevails in virus diseases generally. He later found that the dark green areas of virus-infected sugar beet leaves accumulated more starch than did the light green tissue (17).

Various authors (99) have reported increased starch content in the sereh disease of sugar cane, mulberry-dwarf, curly-top sugar beets, American mosaic of sugar beet, leaf-curl of raspberry, Arricciamento disease of *Vitis*, aster yellows, spike disease of sandal, spinach blight, *Vicia* leaf-roll, and in yellow-blight of tomato.

b. Diastase: Variation in the activity of diastase obviously is of great importance, since this enzyme is one of the components of the carbohydrate metabolism in the plant. Since diastase concerns the equilibrium between starch and its hydrolytic products, it could be discussed in connection with either of the components of the system.

The first quantitative data on diastase changes in virus-infected plants (116) showed that mulberry leaves infected with leaf-roll always had increased diastase activity.

Leaves of sandal infected with spike disease contained only about half as much diastase as normal leaves (37). This was true even though the diseased leaves had accumulated almost twice as much starch as healthy leaves. When the leaves of sandal infected with spike disease were re-investigated (123), their diastatic activity was always greater than normal, in direct opposition to previous data (37).

Virus-blighted spinach had a little less diastase than normal leaves (134). Collections made February, 1915, contained about 3%, those made March, 1916, about 10% less. This was true even though the blighted leaves contained very much more starch.

Neger (86) refers generally to the "hydrolytic diastases" as being somewhat less in activity in leaf-roll potato leaves. This author makes the valid suggestion that the diminished diastatic activity might depend on lessened activity of the enzyme rather than on a change in the actual amount of the enzyme itself.

An early paper (141) implied that diastase was less active in mosaic tobacco leaves. Hunger (66) could not verify Woods' data on the destructive action of oxidases on diastase *in vitro* and thereby implied that Woods' opinion that diastase was injured by oxidizing enzymes in the plant was not correct. A more recent paper (35) states that diastase was less in diseased leaves. This diminution was especially great as the disease increased in severity. Others (112) have found that mosaic tobacco leaves exhibited an equal or greater diastatic activity than did normal leaves, and that this increase did not inhibit the usual accumulation of starch. It was suggested (112) that the synthetic action of the enzyme was responsible for the augmented starch.

Dunlap (47) believed that mosaic tobacco leaves were able to hydrolyze starch more readily than normal leaves, as evidenced by an accumulation of soluble sugar. There is no definite relationship established, however, between the amount of sugar and the diastatic activity.

The sequence of changes in diastase in *Nicotiana tabacum* L., variety Wisconsin-Havana, from the time they were artificially inoculated with virus (6) until the plants were two and one-half months old, has been reported. One virus used was a common type similar to Johnson's No. 1 and another was a yellow mutant, called "yellow mosaic", isolated by McKinney. Inoculation caused rapid decrease in diastase, especially with yellow mosaic. Later, the diastase increased. Its activity did not regain a normal value with Johnson's No. 1 virus, but the yellow mosaic-infected plants attained a final diastatic activity of three or four times that of normal leaves.

The preliminary period of decreased diastase (6) was due primarily to a decrease in an unstable form of the enzyme which is the starch-liquefying component of the system. The increased activity, on the other hand, was due to augmentation of the dextrinizing component. No evidence was found that there existed any inhibitor or activator of the enzyme, and hence this observed change depended on actual changes in their amount rather than in their specific activity.

The above work (6) is the finest that has appeared on this aspect of virus physiology. It is particularly interesting because it shows the great importance of the age of infection in determining the condition of the plant. It is not surprising that there is so much con-

flicting data on diastatic activity, since all these data were published before this effect of age was described.

Guinea pigs inoculated with the virus of hoof-and-mouth disease (93) exhibited increased diastatic activity in cell-free lymph and in blood. The magnitude of this increase paralleled the virulence of the virus. Another investigator could not find these changes (94). Milk from cows suffering with hoof-and-mouth disease had a greater amount of diastase, and this increase became greater as the disease progressed (36).

Purified preparations of tobacco mosaic virus were shown (6) to have no diastatic activity. This is in complete agreement with other data (106). Bacteriophage preparations for cultures of *Escherichia coli* were without diastatic activity (115).

Dextrin. The only paper describing the dextrin content of virus-infected plants (47) reported that only about 75% as much dextrin was present in mosaic tobacco leaves as in normal leaves. This is in agreement with Dunlap's belief that starch is more readily hydrolyzed in mosaic-infected plants. In view of the fact that Dunlap is not in agreement with most writers in regard to carbohydrate changes during virus infection and since the age of the plant and the time since infection is so important in controlling these changes, no general statement can be made concerning the fundamental behavior of dextrin during the course of virus infection.

Total Sugar. Data reported as "soluble carbohydrates" may be regarded as equivalent to the total sugar. An attempt (66) to verify the theory of Woods that the stimulated oxidase activity of mosaic-infected plants caused disturbances in carbohydrate metabolism, showed less total sugar in mosaic tobacco leaves than in healthy tissue, contrary to the implications of Woods' theory.

The dark areas of mosaic-infected leaves contained more sugar than the lighter areas (53), although Dunlap (47) found that mosaic tobacco plants contained about half as much total sugar as normal plants. This author believed that the lessened starch content was due to the greater hydrolytic activity, but this could hardly be in agreement with his observation that the sugars were also lessened.

Köck and Kornauth (73) were unable to detect a significant difference in the sugar content of potato tubers produced by leaf-roll-infected plants. A study (32) on the aerial parts of leaf-roll-in-

infected potato plants before tubers had begun to form was carried out in order that the amounts of carbohydrates in the leaves could not be affected by possible disturbances in the storage processes. It was found that the diseased leaves contained more sugar than normal leaves. On another occasion the disease caused the total sugar content to be increased from 10% to about 100% (130). Whitehead (137) found that leaf-roll-infected potato plants accumulated sugar in the leaves at the expense of that in the tubers, and this increase was correlated with increased production of carbon dioxide.

The soluble carbohydrate content of infected grape leaves was studied (89) at various times of day, and it was found that virus infection greatly lessened the amount. At nine o'clock in the morning, before a significant amount of synthesis had occurred, the relative values were as follows: normal leaves, 3.31; infected but yet green leaves, 0.60; and severely infected leaves, 0.41. These great differences became less as the day's synthesis progressed. This indicates that photosynthetic processes were not inhibited by the disease and that utilization or translocation of the sugar caused the differences observed in the early morning.

Virus-blighted spinach grown at the Virginia Truck Experimental Farm (134) in 1915 contained about 25% more total sugar than normal leaves. The material grown during the next season contained almost 250% more. These differences were great enough to impart a sweeter taste to the leaves.

Sucrose and Other Disaccharides.

a. Amount of sucrose: The data reported in this section include those given for disaccharides and non-reducing sugar as well as those that specifically refer to sucrose.

An increased amount of sucrose in mulberry leaves infected by leaf-roll was inferred by Shibata (116). This author reported an increase of invertase which was assumed to be caused by an increased amount of sucrose.

Leaf-roll-infected grape leaves (89) contained considerably less sucrose than normal leaves, although this difference tended to become less as photosynthesis proceeded through the day.

The effect of yellow-stripe of sugar cane on sucrose content is of considerable commercial interest. Lahaina cane grows so well even when infected that many had assumed that it suffered no

serious physiological disturbances as a result of the disease. However, plants from healthy and infected cuttings exhibit a very small and probably insignificant increase of sucrose (81). Growth is with a consequent loss of total yield of sugar in the crop.

The yellow-stripe or mottling disease of sugar cane was again investigated (43) in respect to changes in sucrose content at various periods of growth. It was found that mere mottling of the leaves did not directly affect the sucrose content. However, in advanced stages of the disease the stalks become cracked and cankered. This condition allows fermentation in the adjacent tissue with consequent increase in acidity. This lower pH was the probable agent causing the somewhat smaller sucrose content in the affected tissue. Another study (126) on the effect of yellow-stripe on sucrose content of sugar cane, failed to give consistent results. There seemed to be an increase of about 5%, although this value is of doubtful significance. Sugar cane in the Philippine Islands infected with virus contained definitely less sucrose. In some instances, diseased plants contained 30% to 40% less sugar.

An extended study (134) of effects of spinach blight on the composition of the crop, led to the conclusion that diseased plants contained almost twice as much sucrose as healthy plants. Mosaic disease of tomato produced a lesser concentration of carbohydrates (20). Twenty-three days after inoculation the plants contained only 1.50% of non-reducing sugar, while normal plants of the same age contained 2.12%.

An extensive study (47) on carbohydrate variations accompanying mosaic in tobacco showed that the disaccharides in the leaves were lessened until they were present in amounts about 70% as great as in normal plants. It is significant that this author's data show that there is a stage in which the carbohydrates in infected plants exceed those in normal leaves. For instance, 15 days after inoculation the disaccharide in mosaic leaves was 152 per cent of that in normal leaves.

b. Invertase: Since the amount of sucrose present is related to the activity of invertase, it is interesting to note the changes that this enzyme undergoes as a result of virus infection. It is strange that there are only three papers which mention the activity of invertase in virus-infected plants. This is all the more remarkable in view of the great amount of attention directed to the general carbohydrate metabolism of diseased plants.

A preliminary publication (116) on leaf-roll of mulberry reported a great increase in the invertase activity in infected leaves. Others (112) reported that the tobacco mosaic disease induced no significant change in invertase.

In the most extensive study of this enzyme in virus-infected plants (144), invertase activity of juice expressed from separate leaves at various distances from the point of inoculation was followed at two or three day intervals. There was a preliminary rise in activity which reached a maximum on about the fourth day after the lower leaf had been inoculated. This was soon followed by a depressed activity which reached a minimum on about the eighth day. This minimum was followed by a second rise which reached a maximum at about the fourteenth to the sixteenth day. The magnitude of this second maximum became progressively less in the leaves higher on the stem. It was at this time that these leaves attained the power to infect young plants with the mosaic disease. Following the second maximum, there was a decrease until the invertase activity approached or became less than that of normal plants.

When inoculation of the lower leaf (leaf 1) was made, the upper (leaf 4) leaf was very small, as were also leaves 5 and 6. These uppermost three leaves also reacted similarly. Like the older group of leaves, leaf 4 showed a preliminary maximum on about the fourth day, which was followed by a minimum at about the sixth to the eighth day. Leaves 5 and 6 were not large enough to be studied until the sixth and eighth day, respectively, and consequently the periods of first maximum and minimum were lost. The trend of changes in each case indicated recovery from a minimum, which, as in the older leaves, probably had taken place on about the sixth to the eighth day. A second maximum developed in each of these younger leaves, as in the older leaves, but the magnitude of this maximum was much less and came on about the tenth to the twelfth day, which was about six days earlier than in the older leaves. This second maximum was followed by a period of lessened invertase activity, as in the older leaves, but differed in being considerably lower. These younger leaves showed a belated approach to normal invertase activity. Since the observation lasted only 21 days, it is not known whether the older leaves might also have exhibited this recovery at a later date.

Glucose and Other Reducing Sugars. The glucose fraction of carbohydrates is regarded as including all reducing sugars and other

substances which reduce the copper in Fehling's solution. Since glucose is the predominant reducing substance in plant tissue, the errors introduced by the method of its determination are not likely to be significant for the present discussion.

Spinach blight was accompanied by a decrease in reducing sugar (134). This decrease varied from 20% to 50% when referred to the sugar content of normal leaves. Virus-infected sugar cane in the Philippine Islands contained 2% more glucose than normal cane (3). This value probably falls within the experimental limit of error in the sampling and determinations. Tomato plants 23 days after inoculation had only 80% as much reducing sugar as normal plants (20). The same general condition existed after 51 days. Different levels of soil fertility did not change this relationship.

The leaves of blighted tomato plants contained three to five times as much reducing sugar (108). As the season progressed this difference became somewhat smaller. The stems contained about twice as much reducing sugar, and, unlike the leaves, this difference increased markedly as the season progressed. The roots of diseased plants contained about twice as much sugar throughout the season. The leaves of sandal (68) infected with spike disease contained from 9% to 800% more reducing sugar than normal leaves. The author's tables, however, show that occasionally there were exceptions to this generalization. Mosaic-infected tobacco leaves contained less than half as much reducing sugar as normal leaves (47).

Cultures of agalactia virus caused no change in the glucose of the culture medium (92).

Pentose and Pentosans. The pentose content in leaves of healthy and leaf-roll-infected potato leaves in an average of four determinations showed that the normal leaves contained 0.44%, while infected leaves had only 0.31% (130). The only other reference (47) concerning the effect of virus infection on pentose content, states that healthy leaves contained 0.27%, while mosaic leaves contained 0.21% pentoses on the fresh weight basis.

In so far as the limited data indicate, there is general agreement that virus infection lessens the per cent of pentoses in leaves.

Causes of Carbohydrate Accumulation

Oxidative Destruction of Diastase. The generally observed increase in activity of oxidizing enzymes in virus-infected tissue has

been the basis of much speculation concerning their etiological significance. As early as 1900, the view was expressed (140) that accumulation of starch in infected leaves was due to inhibition of starch hydrolysis with a consequent effect on translocation rather than to any changed rate of starch production. Woods (140) found that preparations of tobacco oxidase were able to inhibit Taka-diastase and malt diastase *in vitro* and concluded that the oxidative destruction of diastase was of prime importance in causing the accumulation of starch in diseased leaves. This view was further supported (141) by injecting preparations of oxidizing enzymes into healthy plants with a consequent appearance of abnormal amounts of starch.

This theory was seriously attacked by Hunger (66) because he could not duplicate the results of Woods concerning the effect of oxidizing enzymes on diastase *in vitro*. Further, he found that the activity of diastase was not always less in diseased leaves. The report of Shibata (116) appeared soon after that of Hunger, and he, too, was unable to verify the original data of Woods. Hunger (66) and Shibata (116) are in entire agreement that *in vitro* experiments do not show that oxidases inhibit the action of diastase, and also that accumulation of carbohydrates is not necessarily accompanied by a lessened activity of diastase.

The objections to the theory that oxidases destroyed diastase was carried further by Grüss (56) who studied in great detail the relationships of these enzymes in potato tubers. Wounding was found to call forth increased activity of oxidizing enzymes which was followed by actual increase, rather than decrease, in the diastase. This was explained by assuming that the decrease in water content of cells adjacent to the wound concentrated the cell substances. The increased concentration of the proper zymogens, easier access to oxygen and higher oxidase activity were supposed to have produced active diastase. The general theory that oxidation activities produced active diastase was quickly taken up by Sorauer (120) who applied the theory to the condition in leaf-roll of potato.

Chapman (34) states that mosaic and allied diseases are caused by excessive oxygenase and peroxidase, but he does not attempt to explain any relationship between these enzymes and carbohydrate accumulation. The significance of oxidizing enzymes was again taken up by Chapman (35). This author states that in mosaic

tobacco leaves, diastase becomes less, probably because of the increased oxidase activity.

The accumulation of carbohydrates was believed by Grüss (56) and Neger (86) to lessen the activity of hydrolytic enzymes involved in their formation. This is the exact reverse of the causal relationship between carbohydrates and digestive enzymes assumed by Woods (140, 141).

Schaffnit and Lüdtke (112) found that both starch and diastase increased in mosaic tobacco leaves and concluded therefore that the inhibition of diastase reported by other authors could have no necessary etiological significance.

The opinion of Freiberg (53) that the disturbed enzymatic activity is the result of the disease rather than its cause seems to be the most reasonable interpretation of the etiological significance of oxidases.

Increased Chlorophyll in the Darker Areas of the Leaf. Although virus infection brings about a general decrease in the amount of chlorophyll in leaves, it is often observed that local areas exhibit increased chlorophyll content which produces a mottled appearance. These darker areas in mosaic-infected tobacco leaves had more intercellular space than did the light colored areas (39). For instance, the intercellular spaces constituted 17.1% of the total mesophyll and 29.8% of the spongy mesophyll, while the light areas contained only 11.4% intercellular spaces in the total mesophyll and 19.9% in the spongy mesophyll. This condition would probably facilitate aeration and consequently photosynthesis. It was also found that the dark areas contained an increased number of cells, that the cells had a greater number of chloroplasts and that the chloroplasts held more chlorophyll. The dark areas in mosaic tobacco leaves were also shown (49) by accurate quantitative methods to contain more chlorophyll *a* and *b*. Schmidt (114) definitely states his belief that the increased concentration of chlorophyll in the darker areas of mosaic leaves was the cause of the accumulation of carbohydrate in these lesions.

There can be no doubt that the greater aeration due to increased intercellular spaces and increased chlorophyll per unit area would increase the rate and amount of photosynthesis, but these are visible symptoms, and the fact is undeniable that starch does accumulate locally before such symptoms appear. Their rôle as a primary factor in carbohydrate accumulation is, therefore, to be doubted.

Decreased Utilization of Carbohydrates. The assumption that carbohydrates might accumulate as a result of their diminished use in protein synthesis was held to be very unlikely (70). Analyses of virus-blighted spinach indicated that the diseased plants did contain a little less protein, but this was explained in a later paper (71) as being due to denitrification of compounds in the plant with loss of nitrogen in the gaseous form. It is difficult to be certain that the plants lost nitrogen in this manner. True and Hawkins (134) suggest that carbohydrates might accumulate because of their lessened use by the plant, although they offer no argument to support this view.

Rosa (108) states that carbohydrates accumulate because growth of diseased plants is slower with consequent diminished use of manufactured foods. The accumulation was no larger than when the growth rate was diminished to a comparable extent by such means as low temperature or nitrogen starvation, although these analogies are of doubtful value. The very close relationship between growth rate and temperature is well known. Within proper ranges of temperature growth rate about doubles for an increase of 10 degrees in temperature. On the other hand, photosynthesis rates exhibit no such sensitivity to temperature because the amount of available carbon dioxide is usually the limiting factor. Under these conditions plants stunted by low temperatures can be reasonably expected to accumulate carbohydrates. In virus-infected plants, it is reasonable to suppose that inability to transport carbohydrate normally would serve as one of the primary factors in lessening the growth rate.

Nitrogen starvation also serves as a doubtful analogy, since such extreme diminution of available nitrogen in the plant would hinder protein formation with resultant conservation of carbohydrates. There is no necessary correlation between this condition and that existing in virus-infected plants.

Thung (130) demonstrated by copious analytical data that accumulation of carbohydrates could not be due to their lessened use in respiration. In fact, an increased rather than a diminished rate of carbon dioxide loss from virus-infected plants is one of the most common symptoms of the disease.

Mineral Relationships. The possible effect of accumulation of mineral ions in virus-infected tissue on the accumulation of carbo-

hydrates was mentioned by Neger (85), but he admitted that this relationship was not clear.

The only other author to suggest such a relationship (61) published the only experimental evidence. Potato sprouts infected with leaf-roll were able to transport all the starch from the leaves in four days in the dark if they were standing in a potassium chloride solution 1% or less in concentration. If the concentration was increased to 3% almost no translocation was observed. The same results were obtained with potassium dihydrogen phosphate and with potassium nitrate.

The work of Hiltner shows that the effect of the salt content of the diseased tissue cannot be ignored as a possible cause of carbohydrate accumulation. There is need for further experiments, especially with the object of discovering possible abnormal changes in the permeability of protoplasm to specific substances.

Lessened Translocation.

a. Phloem necrosis: Accumulation of carbohydrates in leaves has been explained by some authors as an unavoidable condition due to the death, or at least the more or less disorganization of the phloem cells. It has been observed (85) that leaves on shoots of potato plants infected with leaf-roll were not able to lose starch even after three days in the dark. Leaves on normal plants under these conditions were able to transport the major part of their starch content to the stems. Neger (85) observed the necrotic condition of the phloem in the diseased shoots but he believed that this was caused by undernourishment of these cells because the normal rise of sap was prevented by the diseased condition. Therefore, necrosis of the phloem was not a primary cause of lack of carbohydrate translocation but a result of the disease. Neger does not support this view with sufficient evidence to show convincingly that phloem necrosis is not the cause of carbohydrate accumulation in leaves.

In an attempt by Quanjer (98) to classify the various types of leaf-roll diseases of potato, he designates the general condition as "phloem necrosis". Particularly intense forms of the disease were called "leaf-curl" or "curly-dwarf". Very mild forms were called "primary leaf-roll". The progeny from these mildly infected plants were more intensely affected, and this condition was described as "secondary leaf-roll". All these various manifestations were held

to be the result of the death of the phloem cells. In the early stages of primary leaf-roll only the phloem in the upper parts of the plant was affected. As the disease advanced, this necrotic condition spread downwards. There seemed to be general agreement between the amount of starch accumulated in the leaves and the extent of necrosis of the phloem. This author (98) admits, however, that mosaic leaves of potato and tobacco showed some delayed movement of starch even before phloem necrosis was apparent and suggested that some disturbed enzymatic process was involved. But in spite of this, Quanjer did not doubt that phloem necrosis was a condition generally associated with virus infection, and he quotes from various authors to show that this condition exists in *Arbutifolia* and other members of the Malvaceae, coffee, beet, peach, Japanese mulberry, sugar cane, sandal, banana, *Rubus* and *Vitis*.

Thung's (130) study of leaf-roll of potato showed by analytical data that the accumulation of carbohydrates was not due to any lessened rate of respiration, nor to any enzymatic changes, and he concludes that a disturbed translocation was the prime cause of the accumulation.

The mottled leaves on sugar beet infected with mosaic were shown (107) to accumulate considerable amounts of carbohydrates as a natural result of the death of phloem cells. Only rough qualitative tests for carbohydrates were made, but the differences were so great that more exact quantitative data seemed unnecessary. Böning (16) concurs with Robbins (107), since he believes that the healthy areas of leaves of virus-infected field beans assimilate carbon dioxide normally, but that starch accumulates because translocation away from the synthesizing area is hindered. This author (18) believed that the same conditions exist in curly-top sugar beets.

b. Changes in the permeability of protoplasm: The theory discussed above that virus-induced phloem necrosis is adequate to explain the accumulation of carbohydrates is subject to severe criticism. Even Quanjer, one of the most ardent adherents to the theory, admits (98) that mosaic-infected leaves of tobacco and potato sometimes showed an accumulation of carbohydrates *before* any visible evidence of necrosis of the phloem. His interpretation of these instances was that disturbed enzymatic activities were the responsible agents. It will be seen, however, from the following dis-

cussion that although his observations were correct, his interpretation was probably erroneous.

Thung (130) also reported that starch accumulation in potato leaves infected with leaf-roll was apparent often long before phloem necrosis was visible, and he therefore concluded that the inability of the carbohydrate to leave the leaf itself was more important than phloem necrosis in explaining accumulation.

Murphy (84) studied 23 varieties of potato in great detail and discovered that plants from diseased tubers did not show accumulation of starch from May 15 to June 2. On June 3, starch accumulation began to be apparent, although careful examination failed to show any sign of phloem necrosis. This author observed that phloem necrosis appeared very irregularly, but it is especially common in varieties of potatoes particularly susceptible to leaf-roll. Many times, the extent of the necrosis was not sufficient to account for the amount of starch accumulated in the leaves. Further, there was no relation between the area of the first visible accumulation of starch, which was in the mesophyll of the lower leaves, and the place of necrosis, which was in the lower part of the stem. The carbohydrate translocation in the upper very active leaves was normal during this early appearance of necrosis. It is especially significant that carbohydrates did not accumulate just above necrotic areas.

A study of the separate areas in the leaves showed that carbohydrates of all kinds were absent or nearly so from cells nearer the larger veins and along the vascular tissue. The actual place of accumulation of carbohydrates was the palisade tissue, whence they apparently were not movable. This accumulation became so great finally that the physiological disturbance assumed such magnitude that no more synthesis was possible, and in the midst of plenty, the plants finally died from carbohydrate starvation! Rolling of the leaves depends on mechanical distortion of the palisade cells because of accumulation of starch within them. Bolas and Bewley (14) observed that in tomato during early stages of mosaic infection starch was removed from the leaves faster than in normal plants placed in the dark, *except* from the place of infection. These areas were unable to lose their starch by the usual diffusion of its hydrolyzed components, even though they were surrounded by areas of greatly diminished starch content.

It is therefore apparent that some profound change in the physiology of photosynthetic cells themselves is the actual cause of carbohydrate accumulation. It is impossible to describe at present with certainty, the nature of these disturbances. We have already pointed out the improbability of their being enzymatic. It is not impossible that an abnormal and profound change occurs in the permeability of the cytoplasm or of its membranes, and carbohydrates are thus locked within the cells where they are formed.

An especial importance of the localized accumulation of starch in mosaic tobacco leaves was postulated by Holmes (64). He found that the usual iodine staining test for starch could identify localized tissues invaded by the virus long before other symptoms were apparent. In all instances these localized lesions contained a high concentration of virus material as well as starch. There was no evidence that the starch accumulation preceded virus formation, since they were always found to occur together. The data of Holmes is particularly good evidence that carbohydrate accumulation is a cellular phenomenon occurring in the diseased photosynthetic cells rather than a result of phloem necrosis. It is evident that phloem necrosis can aggravate the general condition by being an additional hindrance to carbohydrate movement.

Osmotic Concentration of the Cell Sap

The osmotic relationships of virus-infected plant tissues were observed for the first time (89) in grape vines. The freezing point of sap exuded from diseased cut stocks was lowered from 0.060 to 0.035 degree, while the value for the sap from normal stems was only 0.023 to 0.0058. The juice expressed from diseased leaves showed a lowering of the freezing point of 2.305 degrees, while the value was 2.142 for the juice from normal plants.

The results of a study (69) on leaves from spiked sandal agree fundamentally with those from grape stems. The osmotic concentration of the juice pressed from infected leaves was always greater than that from normal leaves, even though the ash content was lower. Both workers (89, 69) found a lower ash content in the diseased tissues, and they agree that the augmented osmotic concentration was due to an increase in the number of smaller organic molecules. Pantanelli (89) believed these to be salts of organic acids, while Iyengar (69) believed them to be sugars.

NITROGEN METABOLISM

Total Nitrogen

Amount of Nitrogen. The effect of virus infection on nitrogen metabolism was noted very early in the history of this class of diseases. As early as 1902, chemical data appeared (129) showing that mulberry leaves exhibiting symptoms of leaf-roll were very poor in nitrogen. These data were received with little interest, and it was not until eight years later that a more extensive study based on potato plants was published (122). On the dry weight basis, healthy tubers contained an average of 1.48% nitrogen, while tubers from leaf-roll-infected plants contained 2.06%. On the fresh weight basis, tubers from healthy plants contained 0.36% and those from diseased plants 0.48%. The foliage of the diseased plants also had more nitrogen. On the basis of dry weight, the varieties, "Professor Wohetmann", "Up to Date" and "Magnum Bonum", contained a higher percentage of nitrogen in the diseased leaves (42). In some instances this increase was almost 100%. Tubers from diseased plants also usually contained more nitrogen, although some rare exceptions were observed. There was great variation, however, in the response of different varieties, depending on where they were grown. Some localities uniformly produced tubers having more nitrogen in diseased tubers, while other areas called forth the opposite condition. Potato tubers from leaf-roll-infected plants were again shown to contain more nitrogen than those from healthy plants (50), and leaf-roll-infected potato leaves (32) contained a higher per cent of nitrogen on the fresh weight basis, although on the dry weight basis, the per cent of nitrogen was about the same.

A virus leaf disease of grape (89) was associated with less nitrogen in the diseased leaves and branch tips, while the stems contained conspicuously more.

Sandal infected with spike disease always had less nitrogen than healthy leaves. This condition persisted in the material from several different localities (37). In another study of sandal (68), leaves from diseased trees always contained a greater amount of nitrogen. This increase was 20% over normal. Essentially the same results were obtained (103) from study of the stems of spiked sandal, although the differences were larger.

A diminished percentage of nitrogen was noted (70) in the blighted leaves of spinach. The roots of virus-blighted spinach, on the other hand, contained more nitrogen on the basis of both fresh and dry weight. Others (71) found that mosaic-infected leaves of spinach contained less nitrogen than healthy leaves. Yellows of spinach and aster produced a lower nitrogen content in the leaves (45). This study was later extended (46) to include other species, and it was found that mosaic diseases of tobacco, tomato, pokeweed (*Phytolacca decandra*), cucumber and raspberry increased the total nitrogen in the leaves. It should be noted that the per cent of total carbohydrates was simultaneously decreased. On the other hand, yellows of peach, plum, aster and ragweed (*Ambrosia*) lessened the total nitrogen in the leaves but increased the carbohydrates.

Leaves of mosaic-infected tobacco contained much more nitrogen than healthy leaves (5), and sap expressed from the leaves also revealed increased nitrogen content.

One report (20) states that usually there was no detectable diminishing of nitrogen in mosaic-infected tomato leaves. However, the data, obtained 23 days after infection, show a small but probably significant decrease. The healthy leaves contained 2.46% nitrogen, while the diseased leaves contained 2.31%. When the disease had progressed 51 days after the original infection, the leaves usually showed more nitrogen, although this condition was not always observed. Western yellow blight of tomatoes (108) produced a lessened concentration of nitrogen in the leaves but a higher concentration in stems and roots.

The leaves of curly-top sugar b \grave{e} ets contained about 10% more nitrogen (18).

Carbon: Nitrogen Ratio. Considerable attention has been directed toward the carbon: nitrogen ratio during the past few years because of its supposed relationship to reproductive processes. The effect of virus infection on the ratio was first reported in 1926 (20) for mosaic-infected tomato leaves. The carbon: nitrogen ratio was found to diminish from 9.23 in healthy leaves to 8.13 in mosaic leaves. Analytical data in this paper show that this smaller value was brought about by a lessened carbohydrate content and a more or less constant percentage of nitrogen. A study of various species (46) indicates that the mosaic condition generally causes a diminution of the carbon: nitrogen ratio, since the total carbohydrate con-

tent lessened and the total nitrogen increased. The yellows class of diseases, on the other hand, increased the carbon:nitrogen ratio, since the total carbohydrates were increased and the nitrogen content was diminished. The carbon:nitrogen ratio in leaves of leaf-roll-infected potato was significantly higher than in normal leaves (32).

A great many data on the carbon:nitrogen ratio could be assembled from various authors, but the difficulty of ascribing any specific significance to such ratios in the light of recent research would make such a compilation unprofitable.

Cause of Variations in the Amount of Nitrogen.

a. Disturbed translocation: Suzuki (129) very early surmised that the diminished nitrogen content of virus-infected plants was due to inability of the plant to absorb and transport nitrogen compounds from the soil. He observed that addition of nitrogenous fertilizer to the soil was of no avail in overcoming symptoms of nitrogen starvation in leaf-roll-infected mulberry trees. It would have been interesting in this connection if he had determined the relative amounts of nitrogen in the roots and leaves, since this would have given an indication of whether absorption of nitrogenous nutrients or their failure to be transported to the tops was the predominating factor. Rosa (108) believed that inability to transport nitrogenous compounds was a significant factor in causing the depleted nitrogen content in the leaves of tomato infected with western yellow blight.

Researches in virus-infected potato have yielded significant data on the influence of a disturbed translocation system on nitrogen content in certain tissues. During germination of diseased tubers (122) there appeared to be an increased percentage of nitrogen as compared to normal germinating tubers, because nitrogen-containing reserves were mobilized and removed to the developing shoot slower than were non-nitrogenous reserves. Healthy tubers were able to mobilize and remove nitrogenous reserves faster than non-nitrogenous substances. In later stages of growth, the foliage of diseased potato plants, on the other hand, contained a higher percentage of nitrogen because the new tubers being produced were few and small; hence they did not accumulate the elaborated nitrogen compounds found in the leaves. This interpretation seems to attribute the cause to the result. It is more logical to assume that

the newly formed tubers of diseased plants were small because disintegration of the phloem had seriously lessened the food reserves coming to them. This interpretation was proposed also (50) to explain the relatively greater percentage of nitrogen in germinating diseased tubers.

b. Dilution by carbohydrates: Comparison of percentages of nitrogen components in normal and diseased plants might be considered more important than is truly the case. For instance, the accumulation of carbohydrates, on the percentage basis, would automatically lower the percentage of nitrogen by a dilution effect. It is reasonable to assume that this factor alone suggests superficially a modified nitrogen metabolism when actually little or no such modification occurred. Changes in the ratios of various nitrogenous components present much clearer evidence of a modified metabolism than do changes in the mere percentage of individual components.

c. Loss by denitrification: Various authors have suggested that abnormal reducing intensities of virus-infected tissue or of secondary organisms accompanying the disease may lead to loss of nitrogen from the plant in the form of ammonia or elementary nitrogen. A discussion of these phenomena occurs below in the section describing denitrification.

Protein

Total Protein. The term, protein, as used in the present discussion, includes protein compounds variously reported as protein, raw protein, insoluble protein, insoluble nitrogen and true protein. In many instances the authors do not report sufficient details of their analytical procedures to indicate the particular classes of nitrogen compounds determined. Protein sometimes is reported on the basis of the total nitrogen multiplied by the familiar factor 6.25. Other authors base the protein value on the total nitrogen minus the nitrate and nitrite nitrogen. This fraction is often referred to as raw protein. True protein, on the other hand, is a value depending only on the compounds of high molecular weight, as determined by various procedures.

Raw protein was shown (73) to be considerably increased in potato tubers formed by leaf-roll-infected plants. The protein in the leaves, on the other hand, was significantly diminished. Doby

(42) also investigated leaf-roll-infected potato plants and found on the basis of fresh weight that the tubers from infected plants sometimes contained less and sometimes more protein. On the dry weight basis, irregular results were also obtained, although in most instances more protein was present in the diseased tubers. The amount of protein in the leaves was also variously affected.

Leaves of virus-infected grape (89) contained only about two-thirds as much protein nitrogen as healthy leaves. The normal tissues had 3.05% protein nitrogen; diseased leaves only 1.89%. The same qualitative differences existed in the tips of the branches and in the stems, although the differences were smaller in magnitude.

Freiberg (53) was uncertain concerning the protein relationships in mosaic diseases. He states that there probably is a slight increase of protein in the light areas. It is apparent, however, that the numerical values are changed relatively by the accumulation of carbohydrates in the darker areas, even though the actual amount of protein may have been unchanged.

Mosaic spinach leaves were shown (71) to contain less protein nitrogen on the dry weight basis. The protein nitrogen was a larger per cent of the total nitrogen in the diseased leaves, thought to be due to a loss of some of the simpler nitrogenous compounds. The same authors (72) found that mosaic-infected cabbage leaves, unlike spinach, contained more protein nitrogen, but like spinach, the protein nitrogen was a higher per cent of the total in the diseased leaves. This was again attributed to diminution of some simpler nitrogen-containing compounds in the plant. This phenomenon will be discussed in a later section of the present paper.

Rosa (108) assumed that protein nitrogen decreased in western yellow blight-infected tomato leaves because the soluble nitrogen increased in all parts of the plant. No data were given, and while Rosa's assumptions are probably correct for his method, this is not necessarily true in every case.

Nucleoprotein. The nature of the virus particles producing mosaic has been investigated (8) by means of X-ray technique. The juices from diseased plants yielded a protein fraction composed of liquid crystals. These chemical bodies were entirely absent from normal plants. The clarified sap from diseased plants contained five to ten times as much protein, and this protein was different

from that of normal plants, as shown by different solubilities during purification.

The proteins of healthy plants were more variable. Sometimes nucleoprotein could not be found. Occasionally, however, a two-thirds saturated solution of ammonium sulfate did yield a protein precipitate from clarified healthy juice. This protein contained about 1% phosphorus and also some carbohydrate. This nucleoprotein is not identical to the virus protein, which is also a nucleoprotein, since the virus material can be separated from it. The nucleoprotein from healthy plants is less stable and often disappears from extracts after a few precipitations with acid or ammonium sulfate. Further, proteolytic enzymes do not affect the phosphorus content or infectivity of the virus protein, but these enzymes do destroy healthy nucleoprotein.

The possible nature and structure of active virus particles are beyond the scope of the present review, but it is interesting to note (8) that the crystalline or pseudo-crystalline nature of the virus nucleoprotein may not necessarily eliminate this toxic agent from the class of living organisms. In prepared solutions, the virus particles aggregate and lie parallel, but the adjacent particles do not bear the same relationship to each other as would exist in true crystals. Three-dimensional organisms could also aggregate themselves to form these "crystals", and there is no inherent reason why particles, either truly living or at least possessing many characteristics of living organisms, could not pack into a three-dimensional lattice. There is no essential incompatibility between such crystalline states and the attributes of living tissue. However, only two-dimensional patterns actually have been demonstrated in experimental preparations.

Enzymes.

a. Proteases: Proteolytic enzymes have received but little attention. Schaffnit and Lüdtke (112) were unable to demonstrate pepsin-like enzymes in healthy or mosaic tobacco plants, since gelatin was not attacked by preparations from their material.

Preparations of tobacco mosaic particles in a concentration of 5% in respect to the protein virus were tested (106) for their ability to attack fibrin. All tests were negative.

b. Papain: Papain-like enzymes could not be detected with certainty (112) in normal tobacco leaves, nor in those infected with mosaic disease.

Soluble Nitrogen

Total Soluble Nitrogen.

a. Amount: Investigations (42) on potato tubers from leaf-roll-infected plants indicated that on the fresh weight basis, the total water-soluble nitrogen was either unchanged or slightly smaller in diseased tubers. The same qualitative differences existed with respect to insoluble and total nitrogen. On the dry weight basis, there was either no significant change or slightly more in the diseased tubers, while the insoluble nitrogen still remained less in the diseased tubers. This difference in the relative amounts of soluble nitrogen when calculated on fresh and dry weight bases could not be due to a mere increase in succulence of the diseased tissue, as might first appear, since the relative magnitude of these nitrogen fractions are reversed on dehydration of the tissue. The ratio of insoluble nitrogen to soluble nitrogen on the dry weight basis in one variety was 1.15 in healthy tubers and only 0.92 in diseased tubers, which indicates that soluble nitrogen was a relatively larger part of the total nitrogen in the diseased tubers. The tubers were ripe when these data were obtained.

Non-protein nitrogen in virus-infected grape leaves was always less than in healthy leaves (89). The same relationships existed in stems and tips of the branches. The ratio of protein to non-protein nitrogen was 1.91 in infected leaves and 3.75 in normal leaves.

Western yellow blight of tomato profoundly affected the amount of soluble nitrogen in all parts of the plant (108). This was particularly true of leaves. For example, healthy leaves contained about 0.09% of soluble nitrogen on the basis of fresh weight, while blighted leaves showed 0.111 to 0.155%. The ratios of insoluble to soluble nitrogen were profoundly affected. The first sample collected on June 8 showed in healthy leaves a ratio of 5.7 and in diseased leaves 1.6. By July 24, the values had become 5.5 for normal leaves and 2.9 for diseased leaves. On June 8, healthy roots had a ratio of insoluble to soluble nitrogen equal to 1.9, while the value for diseased roots was only 0.8.

b. Cause: The total water-soluble nitrogenous components in plant tissues include a great number of different substances, some of

which have no well known physiological relationships to each other. Consequently, there is greater difficulty in applying a physiological interpretation to variations in this vaguely defined class of compounds than to insoluble protein. One may assume that variations in soluble nitrogen are brought about by changes in the rate of absorption of nitrogenous nutrients from the soil. On the other hand, changes in the rate of protein synthesis might also cause the accumulation of variable amounts of the soluble intermediate compounds. The present author believes that in most cases the latter assumption is the more probable.

It will be observed from the preceding data that there is generally a lower protein: non-protein nitrogen ratio in virus-infected plants. This is apparent not only in leaves but in storage tissues such as potato tubers, which indicates that all tissues lose some of their ability to synthesize insoluble protein from soluble precursors.

Since translocation of soluble intermediate compounds as well as protein synthetic ability are both lessened by the disease, it is necessary to consider at all times which of these processes is likely to be the limiting factor when an interpretation of these ratios is attempted.

Compounds Containing Reduced Nitrogen.

a. Nicotine. It is surprising that so much chemical work has been done on virus-infected tobacco plants, and yet only a single observation has been reported on the effect of this disease on the nicotine content of the leaves. On the dry weight basis, healthy leaves contained 0.96% and mosaic leaves 0.92% (5). The tissue studied showed a conspicuous increase in total nitrogen in the leaves in spite of the very small effect on the nicotine content.

b. Peptides.

1. *Amount:* It has been found (70, 71) that mosaic of spinach brought about more peptide nitrogen in the tissue. It was not possible (72) to find a similar condition in cabbage leaves where, on the contrary, there was a smaller percentage of peptide nitrogen in the oven-dry leaf tissue. Healthy leaves contained 0.60% while diseased leaves contained only 0.49%. The ratio of peptide nitrogen to total nitrogen did not vary significantly, but its ratio to the water-soluble nitrogen was significantly altered. For instance, in normal leaves the peptide nitrogen was about 18% of the total

soluble nitrogen, while in diseased material it was 20% to 26%. These ratios indicate that some component or group of components in the soluble group of nitrogenous compounds was diminishing under the influence of the disease to a greater extent than were the peptide compounds. The actual data support this interpretation, since the peptide nitrogen was lowered to 82% of normal, while acetamide (ammonia included) nitrogen was lowered to 46% and nitrate nitrogen to only 20% of normal. A possible explanation of why certain lower forms of nitrogenous compounds are so much less in amount is suggested at the end of the section describing the changes in oxidized forms. That diminution of the amount of certain soluble nitrogenous compounds did not become a limiting factor in protein synthesis is indicated by the direct observation that the percentage of protein was significantly greater in the diseased plants.

2. *Enzymes*: Since peptones may be regarded as peptides of high molecular weight, it is interesting to note here that no difference has been found between peptone-splitting enzymes in normal and in mosaic-infected tobacco leaves (112).

c. Amino Acids.

1. *Amount*: The total amino acid nitrogen content of plant tissues obtained by the usual procedures includes the nitrogen present not only in amino acids, but also that in ammonia, and varying proportions of the nitrogen in amides, peptides and amines, depending on the details of the analytical procedures.

Some have concluded (112) that mosaic tobacco leaves contained amino nitrogen to the same percentage of the total nitrogen as did normal leaves, even though the diseased tissues had a little more nitrogen. Determination of amino nitrogen in plant tissues is at best a procedure subject to serious technical difficulties. The difficulty of obtaining comparable extracts, and the uncertainty of the exact nature of the compounds yielding nitrogen by the usual Van Slyke procedure was admitted (112), and the authors held considerable doubt as to the validity of the small differences which they observed. Careful examination of their data suggests that they perhaps were too modest in nullifying the significance of the differences which they observed. They determined the total amino nitrogen in healthy and mosaic tobacco leaves at intervals of three to

six days. In five of the seven determinations, the amount of amino nitrogen per milliliter of expressed sap was higher in the diseased plants, in several instances almost 100%. In only two instances were the values almost identical to those obtained from normal leaves. The ratio of amino nitrogen to total nitrogen was greater in four of the seven determinations. Despite the uncertainty of the data, it seems probable that the total amino nitrogen increases in amount in the mosaic-infected plants, and also that the total amino nitrogen is a greater fraction of the total nitrogen.

The ninhydrin test on the juice from stems and leaves of diseased potato plants gave a positive reaction (13), while normal plants reacted negatively. Since this reagent produces a blue color in the presence of a substance containing a carboxyl and an amino group attached to an aliphatic radical, we might assume that amino acids were present only in diseased tissue. It should be held in mind that this test also is positive for peptides, peptones and some proteins. Quantitative determinations (13), according to direct titration of amino acid by the method of Sorenson, showed that amino-acid nitrogen increased in the stems of potato, variety Klara, from 1.9 milligrams per hundred grams of healthy fresh stem material to 27.4 milligrams in the diseased plants. The variety, Echnacher, contained no amino nitrogen in normal stems, while diseased plants contained 16.3 milligrams per 100 grams fresh weight. The variety, Vesta, showed an increase from 4.21 to 42.1 under similar conditions.

2. *Cause*: There appears to be serious disagreement in the literature concerning the effect of virus infection on the amount of amino acid nitrogen. If this fraction increases, especially in relation to the total insoluble or protein nitrogen, it seems probable that the diseased plants have lost some of their ability to synthesize amino nitrogen compounds into more complex molecules. If, on the other hand, this fraction appears to decrease, there is the possibility that the nitrous compounds produced by the intense reducing systems described in the section below dealing with denitrification of oxidized nitrogen may be effective. These probabilities need not be considered incompatible.

d. Amides.

1. *Amount*: It was found (72) that the mosaic disease of cabbage produced a greatly lessened concentration of amide nitrogen. The

method used included ammonia along with the amide nitrogen. On the basis of oven-dry material, the diseased condition decreased the amount to about 40% of normal. Of greater importance are the data showing that the percentage of this fraction in relation to total nitrogen dropped from about 18 to about 10. Its relationship to the total soluble nitrogen was also disturbed. In normal tissue, amide nitrogen represented about 25% of the total soluble and only about 20% in diseased tissue. This indicates that the amide nitrogen (plus ammonia) was diminished to a greater extent by the disease than were other types of soluble nitrogenous compounds.

2. *Desamidases*: The desamidases form a group of enzymes which act on various amides, such as acetamide, oxamide, benzamide, *etc.*, to release ammonia from the amide group.

3. *Asparaginase* acts upon the amide asparagine, a very important component of the nitrogen metabolism system of plants. The effect of virus infection on the activity of this enzyme has not been observed. Attempts to detect its activity in preparations of tobacco mosaic virus resulted in uniformly negative results (106).

4. *Urease* acts upon urea to release both amide nitrogen groups as ammonia. The relationships of this enzyme to the changes involved in virus infection are unknown. Its presence could not be detected (106) in purified preparations of tobacco mosaic virus.

e. Ammonia.

1. *Amount*: Ammonia has been found in curly-leaf-infected sugar beets (15). Normal tissue was free from this substance. Mosaic tobacco leaves were similar to the diseased sugar beets in this respect. Blighted spinach leaves had a higher percentage of ammonia (70), and mosaic tobacco leaves contained about 5% more than normal (71). The ammonia was a greater fraction of the total soluble and of the total nitrogen.

2. *Cause*: A discussion of possible causes of increased ammonia content in virus-infected plants is in the section describing denitrification. The greater proportion of ammonia in respect to the total amino acid and to the total soluble nitrogen suggests the possibility that diseased plants are less able to synthesize ammonia into higher forms of nitrogenous compounds.

*Compounds Containing Oxidized Nitrogen.**a. Amount.*

1. *Nitrate*: Mosaic spinach leaves contained only about one half as much nitrate nitrogen as normal plants (71). This fraction also was a very much smaller part of the total nitrogen and of the total soluble nitrogen. This indicates that nitrate was disappearing from the tissues faster than can be explained on the assumption of normal nitrogen metabolism. The authors (72) found these relationships even more prominent in mosaic cabbage leaves.

2. *Nitrite*: Nitrite was present in sugar beets only when infected with curly-top and in tobacco leaves only when infected with mosaic (15). Nitrite was in spinach and cabbage leaves only when they exhibited symptoms of virus infection (71, 72).

b. Denitrification. The evidence is fairly conclusive that organisms appearing in virus-infected plants as secondary invaders profoundly affect the nitrogen metabolism of the latter. *Bacillus dianthi* was regularly in all parts of sugar beets affected with curly-top (118, 119). Bonquet (15) described *Bacillus morulans* as a new species which he always found in the diseased phloem of curly-top-infected sugar beets. This organism was shown by *in vitro* experiments to be able to reduce nitrate to nitrite and to ammonia. In fact, this author was able to detect ammonia and nitrite in his diseased plants only when this secondary invader was present. A species of *Streptococcus* also accompanied the tobacco mosaic (15) and was able to reduce nitrate to nitrite and ammonia. These substances were present only when bacterial infection was observable. The general nature of the effects of bacterial infection was further demonstrated by the fact that potato plants contained nitrite only if the tissues were infected with *Streptococcus solani* (15).

This reducing power of the accompanying organisms could be regarded conceivably as an aid to the plant, since the absorbed nitrogen is of no use in synthesis until it has been reduced. But the possibility that bacterial reduction might lead to a loss of nitrogen from the plant was inferred by Bonquet (15), since he suggested that such reduction could lead to a condition of nitrogen starvation of the plant. Coleman (37) apparently agreed with this interpretation, since he quotes the data of Bonquet (15) in his report on the diminished nitrogen content in leaves of spiked sandal. He did not, however, attempt to demonstrate the presence of secondary infectious organisms.

It was assumed (70) that abnormal denitrification occurred in virus-blighted spinach and that this was the primary cause of the lessened total nitrogen and acid amide nitrogen and the increase of ammonia. The authors were the first to actually state that the pathological reducing power of the diseased tissues could lead to a loss of elementary nitrogen, although they did not attribute this power specifically to accompanying organisms. Others (71) applied this interpretation to their data from mosaic-infected spinach.

In a mosaic disease of cabbage, data secured were also interpreted on the basis of an abnormal reducing power of the tissue (72). This paper is unique in its suggestion that the actual loss of nitrogen from the plant was not due to reduction of nitrate and nitrite to elementary nitrogen by secondary bacterial invaders, but to the reaction between the accumulating nitrite ion with amino groups in various types of organic compounds. Neither of these mechanisms for the loss of nitrogen is impossible. At least, it would be difficult to show by experiment that they did not occur. Yet it seems more probable that a lessened nitrogen content is due to a lessened rate of absorption or translocation, as described in the section above which noted possible causes of an altered total nitrogen content.

MINERAL METABOLISM

Total Ash

Amount. Virus-infected potato tubers lost weight during germination at about the same rate as did healthy tubers (121). The inorganic salts were not removed as rapidly, however; hence the germinating diseased tubers always exhibited a higher per cent of ash. The fact that organic reserves were removed faster than inorganic salts from the diseased tubers is interesting in the light of current opinion that organized food moves in the phloem and salts in the xylem. The phloem, rather than the xylem, is more commonly injured by virus infection, and consequently one might assume that the organic reserves and not the mineral components would be removed faster from the tubers. These data were verified (73) and observations were extended to other parts of the plant. In many varieties of potatoes, the tubers of diseased plants contained more ash. The leaves, however, always had less ash, and the roots were variable in this respect.

A later study (42) was not as conclusive as those mentioned above. Only when the data were calculated on the basis of fresh

weight, were they consistent. When so calculated, the daughter tubers from infected potato plants always had less ash than normal tubers. This is not inconsistent with the findings of other students, since the ripe diseased tubers always have conspicuously less dry substance. When the data were calculated on the dry weight basis, they were very irregular. Data on the leaves during growth also were so variable that no significance can be assigned to them. The problem of the effect of leaf-roll conditions on the quality of potato tubers was again approached (50) and previous data were verified. Diseased tubers, on germination, remained richer in salts than normal germinating tubers because the mineral components were removed from the tubers less rapidly than were the organic reserves.

Virus-blighted spinach contains less ash in the leaves but conspicuously more in the roots than normal plants (132), and leaves of mosaic tobacco leaves have less ash than normal leaves (5).

Grape leaves infected with virus contained only about 70% as much ash as normally (89). Spike disease of sandal was shown to induce less ash than is in normal leaves, in some instances as little as one third (37, 68) of the normal value. The average of data obtained over the entire season in sandal stems showed a little less ash than in normal stems. The differences were not as conspicuous as those between leaves from normal and spiked plants (103).

Cause. The above data clearly indicate that virus diseases generally lessen the amount of salts that reach the leaves. Further, there is an inhibition in the movement of salts from storage tissues, as during germination of potato tubers. The most interesting data are those showing that there is actually accumulation of salts in the roots of diseased plants, even though the upper parts, especially the leaves, are conspicuously poor in ash components. It is not to be expected that absorption of soil nutrients proceeds in diseased plants exactly as in normal plants, yet it is evident that the primary factor causing changes in the ash content of various plant parts is the inability of the vascular tissue, xylem as well as phloem, to function normally.

Individual Ash Components

Metallic Components.

a. Calcium: The relative amounts of minerals in virus-infected plants was altered as well as the absolute amount of ash in the

tissue (96). Leaf-roll of potatoes caused a lessened calcium content in developing daughter tubers because this substance, more than the other ash constituents, tended to remain in the leaves. Naturally, the leaves would then tend to contain a higher ratio of calcium to total ash, although no such suggestion was made by this author. It should be noted here that it was assumed (96), probably correctly, that salts move downwards from the leaves as well as upwards from the roots.

A complete analysis of mature sandal leaves (37) showed that those exhibiting symptoms of spike disease contained only from one half to one eighth as much calcium as normal leaves. The ratio of the calcium to the total ash decreased from about 0.34 in normal leaves to 0.09 in diseased leaves. Other analyses (68) of leaves from spiked sandal showed even a smaller per cent of this substance. In healthy leaves, the ratio of calcium to total ash was 0.33 and in diseased leaves, 0.19. The stems of spiked sandal contained a smaller percentage of calcium than normal stems (103). The ratio of calcium to total ash was much smaller in the diseased stems.

One study (53) revealed no significant changes in the calcium content of mosaic tobacco leaves, while others (132) showed that calcium was always higher in both the tops and roots of virus-blighted spinach, the ratio of calcium to total ash increasing in the tops from 0.06 to 0.11 and in the roots from 0.01 to 0.07. A virus disease of grapes (89) induced a slightly larger ratio of calcium to total ash of the leaves. The differences were very small, and there was also a slight increase of this ratio in the stem tips of the diseased plants.

The foregoing data of the several authors present a variable aspect. Only in spinach did virus infection increase the percentage of calcium. The ratio of calcium to total ash is greatly increased because not only was the amount of calcium increased but the total ash was decreased. The spike disease of sandal lessened the percentage of calcium in the leaves. Even though the total ash was also greatly diminished, its diminution was relatively less than that of calcium; hence the ratio of calcium to total ash lessens. The significance of these facts is discussed in the section below on differential ion absorption. There is also a probable relation of the lessened calcium to the permeability of the mesophyll cells which has been discussed in the section describing the causes of carbohydrate accumulation.

b. Magnesium: The leaves of spiked sandal contained less magnesium than normal tissue (37). This reduction was very much less, however, than was that of calcium. The ratios of magnesium to total ash increased markedly, in direct opposition to the changes in the ratios of calcium to the total ash in the same tissues. One set of samples showed an increase from 0.07 in normal leaves to 0.78 in diseased tissue. Another set from another locality showed an increase from 0.07 to 0.10. The changes in the relative amount of magnesium in the ash are seen to vary in opposition to the changes in the ratios of calcium to the total ash in the same tissue. A set of material from Bangalore contained an abnormal amount of ash constituents, largely calcium, and these leaves had less magnesium than normal. Absorption of calcium by the diseased plants dropped to one eighth of that in normal leaves from the same area. This greatly disturbed calcium balance, even by the normal plants from this area, profoundly affected magnesium absorption. This material showed an increased percentage of magnesium in the diseased leaves, although the ratio of magnesium to total ash diminished from 0.34 in normal tissue to 0.10 because of the exceptionally large amount of calcium present. This is a fine example of the importance of changes in the differential permeability of plant cells affected by disease.

A significant change in the magnesium content of mosaic tobacco leaves has not been observed (53). Blighted spinach leaves sometimes had more, sometimes less magnesium than normal leaves (132). The roots of diseased plants always had about twice as much magnesium. The ratios of magnesium to total ash increased always in the leaves and especially in the roots. The ratio of magnesium, calculated as MgO , to the total ash of leaves of virus-infected grapes was shown (89) to diminish greatly. One set of analyses displayed diminution from the normal value of 0.0659 to 0.0493. The ash of stem tips also showed a decrease in this ratio, from 0.0848 in normal to 0.0563 in the infected samples.

Available data are so meager that positive conclusions are difficult to obtain. The data do indicate, however, that the effect of virus infection on the percentage of magnesium is variable, yet the ratio of magnesium to the total ash is generally increased in all parts of the plant. This is especially marked in the roots, which indicates a profound change from the normal differential ion absorption balance.

c. Potassium: It has been suggested (96) that potassium seemed more mobile within leaf-roll-infected potato plants than calcium, and consequently, relatively more potassium than calcium reached the developing potato tubers. This might also explain the increase in the ratio of calcium to the total ash in those tissues.

Leaves of spiked sandal (37) contained less potassium than normal leaves. The ratio of potassium to the total ash was always increased by the disease, although to a very much less extent than were calcium and magnesium. Absorption of this ion was less disturbed by virus infection in this species than were the other major metallic nutrient ions. The potassium content of spiked sandal leaves was again investigated (68) and shown to be greatly diminished as a result of infection. The ratios of potassium to the total inorganic constituents did not change significantly. Sometimes this value was a little lower, sometimes slightly higher than normal. These data agree with other data (37) and indicate that absorption of potassium was less disturbed by virus infection in this species than in spinach. In stems of spiked sandal the disease slightly increased the ratio of potassium to the total ash (103).

The mosaic disease of tobacco had but little effect on potassium content (53). Spinach leaves showing symptoms of virus disease had much less potassium (132). The diseased roots, on the other hand, contained much more than normal roots. In the leaves the ratios of the potassium to total ash were lessened by the disease, while in the roots these ratios increased to three or four times the normal value, which indicates a more profound disturbance in the potassium balance in this species than in sandal. Other data (89) showed a slight increase in the ratio of potassium, calculated as K_2O , to the total ash of the virus-infected leaves of grapes. The ash of the diseased stems, on the other hand, exhibited a somewhat smaller ratio.

d. Sodium: Only a single observation of the effect of virus diseases on sodium content has been reported (132). Leaves of blighted spinach contained only about 85% as much as normal plants. The roots also revealed conspicuously less. In normal leaves, the ratio of sodium to total ash was 0.10, while in blighted plants the value was 0.11. The relation of sodium to total ash appears therefore to vary reciprocally with potassium. This condition is very important in roots. Normal roots contained 1.65% sodium,

calculated as Na_2O , while diseased roots had only 0.52%. This great reduction in the absolute amount, when compared to the accompanying great reduction in total ash, shows that sodium was being absorbed relatively less by the diseased roots, while potassium was being absorbed in relatively greater amounts. For example, the diseased roots exhibited a ratio of potassium to total ash of only 0.05, while in normal roots the ratio was 0.25! The reciprocal relationship between absorption of sodium and potassium is well demonstrated by the ratio for potassium to the total ash being 0.35 in the infected roots and only 0.14 in the normal roots. This is a fine example of how profoundly the metabolism of plants can be affected by secondary effects of disease. It also illustrates how difficult it is to separate cause from effect in interpreting pathological conditions.

e. Iron: Spike disease of sandal caused a lower iron content in mature leaves in most instances reported by one investigator (37), while another (68) reported no significant difference. The small differences in the latter appear to be within the limits of experimental error.

There was no significant change in the iron content of mosaic tobacco leaves (53). Data from virus-infected grape plants show that there were relatively larger amounts of iron per unit of total ash in the leaves and also in the tips of the stems (89).

Leaves of virus-infected spinach contained significantly less iron (132), the roots much more. The material grown at the Jones farm at the Virginia Agriculture Station contained 0.062% iron in the normal roots and the diseased roots 0.093%. Material from the Whitehurst farm had 0.069% iron in normal tissue and 0.152 in diseased roots. The ratios of iron to the total ash of the leaves increased from 0.0051 in normal to 0.0056 in the infected tissue. The roots had a ratio of 0.0093 in normal and 0.0096 in diseased material; hence there is a small but consistent change in the differential absorption of iron in this species.

It is evident from the data that iron tends to accumulate in the roots in many instances. It is not impossible that an altered permeability of the cytoplasm to iron may be a factor in producing the localized chlorosis observed in many types of virus disease.

f. Manganese: An interesting reciprocal relationship between manganese and iron in the leaves of blighted spinach plants ap-

peared when the data were calculated on the basis of total dry weight (132). This relationship disappears when the data are calculated on the basis of total ash content. For example, the leaves showed an increase of 0.031% in normal tissue to 0.043 in diseased, calculated as Mn_3O_4 . The iron content decreased from 0.122% to 0.102%, calculated as Fe_2O_3 . Another set of samples showed an increase of manganese from 0.021% in normal to 0.045% in diseased leaves. There was always an increase in the amount of manganese in the roots, although in both sets of data there were also increases in the amount of iron.

The ratios of manganese to total ash increased significantly in both leaves and roots. The same qualitative changes were observed in the iron content, although the differences were much smaller in magnitude.

g. Aluminum: The only data bearing on the effect of virus infection on aluminum content of plants show that the tops of blighted spinach plants contained sometimes more and sometimes less aluminum than normal tissue (132). The ratio of aluminum to total ash always increased, in one instance from 0.13 to 0.23, in another from 0.19 to 0.21. The roots of blighted spinach showed no consistent change in aluminum content and in one case the disease caused an increase from 0.18% to 0.24%. In another, there was a decrease from 0.18 to 0.19. The ratios of aluminum to total ash, however, always were lessened in the roots by the disease, in one instance from 0.0269 to 0.0251, in another from 0.0237 to 0.0106.

Acidic Components.

a. Sulphur: No significant change in sulphur content as a result of mosaic in tobacco was found (53). Others (132) reported that blighted spinach leaves contained only about one half as much sulphur as normal leaves. The ratio of sulphur to total ash content was also very much less, being reduced from 0.0314% to 0.0187%. This shows that accumulation of sulphur in the leaves was more inhibited than was salt accumulation in general. The roots reacted to the disease variously. In some instances there was more and in some less than in normal roots, and the ratios of this substance to total salts was also unpredictable. There was a great decrease in the ratio of sulphur in the ash of virus-infected grape leaves (89).

b. Phosphorus:

1. *Amount:* Mosaic of tobacco induced no appreciable change in the phosphorus content of the leaves (53). Virus blight of spinach, on the other hand, greatly lessened the phosphorus content calculated as P_2O_5 (132). The ratio of phosphorus to total ash was greatly diminished, in one set from 0.0671 to 0.0636, in another from 0.0619 to 0.0405. This indicates that phosphorus, like sulphur, was more inhibited in its accumulation than were salts generally. Diseased roots, to the contrary, contained a greater percentage of phosphorus than did normal roots, although the ratios of phosphorus to total ash in roots showed no definite trend as a result of infection.

Spike disease of sandal also diminished the percentage of phosphorus in the leaves (68). While diseased leaves always contained less phosphorus, the ratios to total ash did not change consistently. For example, one set of data showed that the healthy leaves had a ratio of phosphorus to total ash of 0.082 and the diseased leaves 0.11. Another table yielded ratios of 0.29 in healthy and only 0.045 in diseased material. Leaves from spiked sandal always contained much less phosphorus than did those from normal plants (37). The ratios of phosphorus to total ash were 0.037 in normal leaves and 0.039 in diseased leaves. In another locality the diseased lessened their ratio from 0.065 to 0.062. In a third locality, the disease increased the ratio from 0.026 to 0.050. The erratic qualitative magnitude of the ratios indicates that the disease inhibited general salt accumulation more consistently than it did phosphorus. Stems contained a higher ratio of phosphorus to total ash (103). During one season it averaged 0.0494 in normal stems, but 0.0536 in spiked plants. In another season, the diseased increased this ratio from 0.0182 to 0.0259.

A virus disease of grapes greatly lessened the ratio of phosphorus (89), calculated as P_2O_5 , to the total ash in the leaves. Normal leaves contained phosphorus in the ratio of 0.0604 to the total ash, while diseased leaves had a ratio of only 0.0415. There was an even greater decrease in this ratio in the ash from diseased stem tips.

2. *Phosphatase:* Phosphatase activity has not been detected (106) in preparations of tobacco mosaic virus.

c. Silica: The silica content of diseased spinach leaves always increased (132) as well as its ratio to total ash. The roots also

contained more silica, although the ratio to the total ash was not always greater. There is usually an increased silica content in leaves from spiked sandal (68).

Differential Ion Absorption. It will be noted from the discussion of the effects of virus diseases on mineral absorption that whenever possible the ratios of individual substances to total ash components are given. In most instances these have been calculated from the published data, since few authors have included these values in their original publications. This influence of virus diseases is stressed in the present review because of the obvious importance of an altered selective permeability to soil nutrient ions as a profound secondary character of plant disease. That virus diseases can affect, in this way, the nutrition of plants is of considerable importance in the future development of the physiology of plant disease in general.

Students of permeability will not fail to note that profound changes in these ratios accompany the universal lessening of total salt content induced by virus infection. For example, the magnitude of the ratio of calcium and magnesium to total ash vary reciprocally; *i.e.*, the calcium ratio in the leaves generally diminishes and the magnesium ratio increases. Potassium absorption seems to be less affected by changed selective permeability than other positive ions.

Absorption of negative ions containing sulphur and phosphorus was generally decreased much more than that of other ions. An increased silica content always accompanied the decreased total inorganic nutrient absorption by diseased plants. Its ratio to the total ash was consequently greatly increased.

Discussion of how these altered permeabilities specifically affect the physiology of the plant lies beyond the scope of the present review. Recent developments in the study of the colloidal activity of protoplasm emphasize that these effects are numerous and varied.

WATER RELATIONSHIPS

Transpiration

Pantaneli (89) studied the effect of virus infection on the rate of transpiration of grape leaves. By drawing water-free air over branches enclosed in a chamber and by weighing the transpired water which was collected in a calcium chloride tube, he determined that the rate of transpiration was greatly lessened by the disease. In the light, normal plants transpired 8.65 milligrams of water per

square centimeter of leaf surface, while diseased leaves in the same time lost only 0.73 milligram. The normal rate was therefore 11.8 times that brought about by the disease. When the transpiration rates were calculated on the basis of dry tissue, normal plants lost 448.9 milligrams of water per gram, while diseased material lost only 59.8 milligrams. On this basis, normal tissue transpired about eight times as rapidly as diseased tissue.

In the dark these differences disappeared or even reversed their relative magnitudes. In the light, normal leaves may have lost more water by transpiration because of the greater chlorophyll content and consequently greater absorption of radiant energy. The slightly greater loss of water by diseased leaves in the dark may have been due to their increased respiration or because the diseased protoplasm was unable to retain normal water relationships. While the literature of plant respiration contains ample data to indicate the relationships between transpiration and respiration, there is no conclusive evidence that the abnormal condition of protoplasm is not also an important factor.

Root Pressure

Virus infection greatly reduced the root pressure of grape stocks (89). The volume of water exuded from cut stocks was determined and this value was regarded indicative of root pressure. Normal stocks exuded 284 to 300 cubic centimeters of sap, while infected plants exuded only 40 to 115 cubic centimeters.

Succulence

Dry Weight. From the earliest period in the study of virus diseases in plants, many authors have reported that this group of diseases increased the percentage of dry material and lessened the succulence of the tissue. Leaf-roll of potato increased the dry substance in the tubers from 22.55% to 24.6% (121, 122). Others (73), however, were not able to verify this, and the behavior of a number of European varieties indicated that leaf-roll reduced the dry material in the tubers in most instances, although exceptions were observed (42). It is evident that potato tubers do not respond consistently in this respect.

Leaf-roll of *Syringa* induced an increase of about 100% in the dry weight of the leaves (74) and the same condition was reported (70) for blighted spinach leaves, although the magnitude of the

increase was smaller. These data were later verified (134). Calicoed tobacco leaves (5) had a little less dry material than normal leaves, and appeared exceptional in containing less carbohydrates, contrary to most observations. Curly-top of sugar beets increased the dry weight of the leaves (33) and potato leaf-roll increased the dry substance in the leaves significantly (32). There was also an increase in the dry weight of leaves of mosaic tomato (20) and western yellow blight of tomatoes (108). Spike disease of sandal also increased the percentage of dry substance in the leaves (68).

From the data reported above it is evident that virus infection always increases the percentage of dry substance in leaves. The single exception (5) does not nullify this generality, as will be seen from the discussion below on the causes of this increased dry substance. Potato tubers appear to react variously. Sometimes leaf-roll causes an increase, sometimes a decrease in the percentage of dry substance. The factors affecting accumulation of substances in tubers are so various that this lack of uniform response is not surprising.

Cause.

a. Carbohydrate accumulation: The discussion in an early section of the present review described the almost universally observed increase in the percentage of carbohydrates in virus-infected tissue. This condition itself would tend to increase the per cent of total dry material with consequent diminution in succulence. Changes in the amount of dry matter are probably the most important single factor in bringing about this aspect of the composition of the tissue. This relationship is indicated in an important manner by data (5) showing that calicoed tobacco leaves exhibited a decrease in dry matter, with an increase in succulence. This tissue, however, also was exceptional in that analyses showed lessened amount of carbohydrate.

b. Water absorption: Disturbed water absorption has been reported only for grapes (89), but in so far as this fragmentary evidence goes, it indicates that the virus condition inhibits normal water absorption. The fact that virus-infected stocks exuded less water than normal stocks is significant evidence in this regard.

c. Transpiration: Data presented above show that virus infection greatly decreases transpiration. Unfortunately, the data are

not extensive, though sufficient to show the importance of this phenomenon.

The probable reduced ability of the plant to absorb water might conceivably be balanced by the reduced rate of transpiration and a normal water content maintained. This is a further indication that accumulation of carbohydrates is the most important factor in increasing the percentage of dry substances in the plant.

MISCELLANEOUS SUBSTANCES

Acids

As early as 1903, it was reported (66) that mosaic leaves of tobacco contained less free organic acid than normal leaves, although the data were not verified by later study (57). In no instance did the juice from mosaic tobacco leaves contain less hydrogen ion concentration than healthy juice. In most cases, the infected juice was distinctly more acid. This increased acidity was believed (57) to be at least partly responsible for the lessened activity of catalase.

By suitable dilution, the acidity of separate parts of the plants of *Nicotiana tabacum*, *N. glutinosa*, *N. rustica*, *Datura stramonium*, *Solanum lycopersicum*, *Phaseolus vulgaris* and *Beta vulgaris* was measured by means of the hydrogen electrode. Direct measurement of the anaerobic potential in raw press juice, tissue brew or leaf sections of the green parts gave unlike values. Interfering substances were removed by the centrifuge. When thus prepared, the juice from *Nicotiana tabacum* showed very little difference in acidity in different leaves from the same plant. Virus-infected *Nicotiana tabacum* and *Beta vulgaris* showed acute alkalosis, with an increased buffer capacity between pH 3 and pH 4. Franke's (52) suggestion that this was due to the virus particles themselves is interesting but not adequately supported by evidence.

Almost without exception virus-infected potato plants exhibited a lower acidity of the stem sap (13). This was shown by a change in pH from 5.64 to 5.82 and also by a decrease in titratable acidity. These conditions were not universal, however. In potato leaf-roll a very significant increase in acidity of the press juice was observed from diseased tubers and from other parts of the plant (110).

Acidity of sugar cane juice was not greatly altered by yellow-stripe (43), but when the lesions became cankered, bacterial infec-

tion resulted. The sucrose was fermented sufficiently by these invading organisms to bring about a significant increase in titratable acidity. This increased hydrogen ion concentration became a significant factor in increasing the hydrolysis of the accumulated sucrose. Others (33) agreed that virus infection increased the titratable acid in the juice of sugar cane. This increased acidity was accompanied by smaller amounts of sucrose, presumably because its hydrolysis was catalyzed by the increased number of hydrogen ions.

Both spike disease of sandal (123) and aucuba or yellows disease of tomato (14) always caused greater acidity.

Tannins

Tobacco mosaic lessened the amount of tannins in the leaves (66). It should be noted that Hunger's data based on these tissues did not agree with that of most writers in other respects. For example, the diseased leaves contained less sugar and less acid. It is, therefore, not impossible that his material was also atypical in its tannin content.

Fats and Oils

Raw fat, as reported by most writers, included not only true fats and oils, but all ether-soluble substances. Leaf-roll of potatoes greatly lessened their content of raw fat, and tubers of the variety Znicz exhibited a drop from 2.31% in normal material to 1.68% in the diseased (73). Variety Bojar reacted by a diminution from 2.22% to 1.75%. Other data (42) do not indicate such profound effect. On the fresh weight basis, there were either very small or no differences. On the dry weight basis, the mature tubers appeared to have reacted variously. In five instances there were very slight increases, but these differences fall within a reasonable experimental error. In two instances there was a small decrease. On the other hand, most of the data published by Thung (130) indicate a definite diminution in fat content.

Fiber

The raw fiber content in mature potato tubers was greatly lessened by leaf-roll (73). In one variety the value fell from 22.9% to 14.1%; in another from 17.24% to 14.37%. Other data (42) agree essentially with the above, although a few analyses show

no change in the amount of fiber and in rare cases even a slight increase. The mosaic disease of sugar cane caused a smaller percentage of fiber in the cane (3). Even though the available data are meager, it appears probable that a low percentage of fiber accompanies virus infection.

Anthocyanins

There was an increased amount of anthocyanins in the starch-gorged leaf cells of virus-infected sugar beet plants. The authors believed that the abnormally high starch content was the cause of the increase in these pigments (18).

Oxalate Crystals

There was an increase in the number of microscopically visible calcium oxalate crystals in the darker areas of mosaic-infected sugar beets (114). The author believed that this was due to the releasing of free bases during protein synthesis which were neutralized by oxalic acid. A greater protein synthesis was held to be evidenced by the increased carbohydrate content in the darker areas of the leaf. Sufficient data are not available to disprove this theoretical interpretation. It is well known, however, that free oxalic acid or soluble oxalate do tend to accumulate to an unusual extent in pasture plants growing with exceptional rapidity. There are many cases reported in the literature of animals suffering from calcium tetany due to absorption of oxalic ions into the blood. The relation of oxalic acid to protein synthesis in the so-called "acid" or "ammonia plants" requires further study.

SUMMARY

Respiration

Gaseous Exchanges. Most data indicate that virus infection increases the respiratory rate of plants. Rarely is the rate less, and more rarely has it been reported unchanged. These divergent results are explained by the fact that respiratory rates vary during the period of infection. The rate is increased in the early stages, but a decrease follows until the rate becomes less than normal, or approximately normal. The highest rates occur when the virus first reaches the tissue from the point of infection and it is not related to the accumulation of virus material during progress of the disease.

Oxidizing Enzymes. Oxygenase activity increases as a result of virus infection, although there may be an initial period of lessened activity. In tobacco, the preliminary period of minimum activity occurs at a progressively later stage in the younger leaves. In advanced stages of the disease, the oxygenase activity may drop to normal or less than normal.

Peroxidase also is increased at some stage of the disease, but like oxygenase its activity may be diminished during a preliminary period, and in advanced stages it may fall to normal or less.

The data for tyrosinase are few and conflicting. It is probably that its activity is increased. There are no convincing data on the effect of virus infection on dehydrogenase enzymes.

Catalase activity increases during the early stages of infection. In tobacco, this increase is followed by a progressive decrease.

Carbohydrate Metabolism

Chloroplast Pigments. The darker areas of mosaic leaves contain more chlorophyll, although generally virus infection lessens the amount. The increased oxygenase activity, higher acidity, smaller number of chloroplasts, and higher chlorophyllase activity may be important factors in producing this condition.

Carotene is increased, xanthophyll decreased, although very few data have been published on the effect of virus infection on these pigments.

Carbohydrate. Carbohydrate accumulates as a result of infection. This is probably not due to the destructive action of oxidizing enzymes on diastase, nor to decreased use in protein synthesis, as has been suggested. More probably, carbohydrate accumulates because of reduced permeability of synthesizing cells to sugar. In some instances, phloem necrosis may prevent translocation, although this is not the fundamental cause of carbohydrate accumulation.

Nitrogen Metabolism

Total Nitrogen. The total amount of nitrogen is usually smaller. This might be accounted for on the percentage basis, by the dilution effect of accumulated carbohydrates. More significant, however, is the loss of the ability to transport soluble nitrogen from the roots or from storage tissue. Some authors have suggested that the intense reducing properties of secondary organisms of infection releases gaseous nitrogen or ammonia from the tissue.

Soluble Nitrogen. Generally, there is a lower protein:non-protein nitrogen ratio in the diseased plants, which indicates that the cells lose some of their ability to synthesize protein from soluble precursors. Since translocation of the precursors may also be affected, it is necessary to consider whether disturbance in synthesis or in translocation is affecting nitrogen metabolism. Since these factors are opposite in their effect on the accumulation of amino acids and other forms of soluble nitrogen, it is apparent that there would be a great variation in the amount of these substances in the diseased tissue.

Nicotine. A single author has reported that the nicotine content of tobacco was lowered by virus infection.

Mineral Metabolism

Total Ash. Virus infection lessens the ability of the plant to transport salts. This causes an accumulation of salts in the roots and a reduction in the amount which reaches the leaves. There is also an inhibition in the movement of salts from storage tissue. This is not necessarily dependent on changes in the conductive tissue, although such changes may augment this condition in advanced stages of the disease.

Individual Ash Components. Virus diseases usually lower the amount of calcium in the tissues. This diminution is greater than that of the total ash components, which results in a lower Ca:ash ratio. Magnesium also is lessened, although to a much smaller degree than calcium. The Mg:ash ratio increases, in direct opposition to the Ca:ash ratio, which makes it apparent that absorption of magnesium increases, relatively, as calcium decreases. The absorption and translocation of potassium is less affected by virus diseases than is calcium and magnesium. Generally, there is a little less in diseased tissue. The ratio of sodium to the total ash varies reciprocally with that of potassium. The concentration of iron in the tissues is variously affected. It tends to accumulate in the roots of diseased plants. Manganese frequently varies inversely as iron, although it also tends to accumulate in the roots. The amount of aluminum in the tissue is variously affected, although its ratio to total ash is always increased. Absorption of sulphate and phosphate generally is decreased proportionately more than other ions, which lessens the ratio of these substances to the total ash. Silica absorp-

tion is always greater in diseased plants, which enormously augments its ratio to total ash.

Differential Ion Absorption. Virus infection alters the permeability of the protoplasm in various degrees in respect to different nutrient ions. This is evident, since the ratios of the individual ions to the total ash diverge widely from their original values as the accumulation of total ash components is reduced by the disease. That virus diseases can thus change the nutritional state of the plant by this secondary condition developing during the course of the disease is of great importance in the interpretation of the physiology of plant diseases in general.

Conclusions

There are two major physiological effects of virus infection in plants which may be summarized as follows:

Respiratory activities are markedly increased very early during the course of the infection which corresponds to the period of arrival of virus particles from points of infection. The plant recovers from this initial shock, although the quantity of virus protein continues to increase.

The permeability of cytoplasm or its membranes is greatly altered in respect to soluble substances. This accounts for the accumulation of carbohydrate in the leaves, and the accumulation of nutrient ions in the roots at the expense of tops. Since the changes in permeability vary for different ions, the nutritional state of the plant is more significantly disturbed than the general reduction of the total ash would suggest.

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GEOGRAPHICAL DISTRIBUTION OF FUNGI

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INTRODUCTION

During a sojourn of some years in Manitoba, I became impressed by the large number of genera and species of fungi there in an environment that I had supposed would be unfavorable for them. Diligent collecting, by a few others and myself, disclosed 2,625 kinds, including 98 myxomycetes. It seemed evident that more than 5,000 species must be present (6), though only some 1,200 phanerogams are known to grow there without cultivation, and a hundred of these are introduced weeds or escapes. Green plants, then these fungi, had arrived and established themselves during not more than 25,000 years since the last glacier retreated.

After some thought and analysis I concluded (5) that at least twice as many phanerogams as fungi had been described for the world, but that in any state or country the number of species of fungi is likely to exceed the number of species of phanerogams because of the greater average range of a fungus; that the smaller the area surveyed, the greater is the excess of fungi; that saprophytic fungi generally have wider distribution than parasitic; and that distribution of hosts and substrata, principally flowering plants or their remains, has more influence than climate upon distribution of fungi.

Those conclusions are fairly obvious, but it should be possible to extend them. I now realize that a number of Manitoban fungi were not good morphologic species; that I had given most of my attention to the fungi of temperate regions, and therefore underestimated the effect of climate.

The study of distribution may be approached from various angles, from that of the whole world and all time to that of today's dispersal over one acre. I happened to be present during the arrival of a few fungi in Manitoba (5, 8); those recognised as new were all parasites of cultivated plants; I knew of no sure means of ascertaining when other fungi had reached the Province.

KRAKATAU: DISPERSAL IN OPERATION

The volcanic eruption of 1883 left the islands of the Krakatau group probably sterile, at least as regards fungi. These islands

are now covered with a rich vegetation which has been carefully analysed. During the 50 years following the eruption a total of 263 species of phanerogams and 61 of pteridophytes had been found in gradually increasing numbers. Not all were able to persist, but in 1934, 219 phanerogams and 52 pteridophytes were reported (20). Immigration of fungi and lichens was not followed through the years, but a survey in 1933-4 gave 310 species of fungi, including myxomycetes, in 150 genera (9). Only 13 species of lichens were recorded.

The number of fungi was greater than that of vascular plants and would of course have been greater still if every fungus had been found and identified. The 310 species were grouped as follows: Myxomycetes, 28; Phycomycetes, 4 (all Mucorales, 3 of them species of *Mucor* isolated from the soil); Discomycetes, 10; Pyrenomycetes, 56; Uredinales, 2; Hymenomycetes, 150 (78 Polyporaceae); Gasteromycetes, 9; Fungi Imperfecti, 51 (34 Hyphomycetes).

Krakatau is about 40 kilometres from both Java and Sumatra. Boedijn found that a tree trunk with mycelium might occasionally be washed ashore and later produce spores, but he considered that nearly every fungus had arrived by air; for 50 years spores had settled on the islands, though none could develop unless its host or substratum had arrived. Twelve species are described as new from the Krakatau group, but, as Boedijn states, it is very unlikely that any of these is really endemic, *i.e.*, "evolved" during the last 50 years.

Considerable search for lichens yielded only 23 collections belonging to 13 species, all epiphytes. None was found on rocks or soil, none on any of the islands except Krakatau. Lichens constitute most of the flora of the exposed rocks of Antarctica (33), but evidently these organisms rarely managed the trip from Java, Sumatra or elsewhere to Krakatau.

AGENTS OF DISPERSAL

We are here concerned particularly with the distribution of fungi over wide areas; but any agency that permits their spread may be considered. Fungi are estimated to have appeared upon the earth a couple of hundred million years ago, so they have not had to hurry. Many species now utilize more than one means of dissemination, *e.g.*, ascospores by air, conidia by contact.

Dispersal with the aid of water and air has been analysed by Dobbs (19) and by Mason (28). Craigie (14) has reviewed aerial dissemination of fungi pathogenic to higher plants.

Air. It is recognized that light, powder-like seeds afford more rapid transit than plumed or winged seeds (36). Pteridophytes, with spores only about 30–50 μ in diameter, travel farther and faster than seed plants. Most fungi have even smaller spores; a spherical spore 5 μ in diameter has a volume of only 65 cubic microns, in contrast to 65,000 for such a spore 50 μ wide.

It is obvious that a slight current of air will support most fungus spores, provided they can escape from their sporophores. Nature has provided for this (11, 25): most basidiospores are launched by forcible abjection, most ascospores by ejection, and many other fungi, *e.g.*, dry-spored Moniliales (28), Gasteromycetes, rusts and smuts, have powdery spores to be picked up by a breeze. Furthermore, most fungi produce spores in vast numbers. Viable spores have been caught thousands of feet up in the air. However, a spore is not likely to get across an ocean, or to be still alive if it does.

Even though a fungus has no device for getting its spores into the air, or though they fall to the ground, the wind may pick them up with dust.

Animals. Many fungi, such as Phallales, Sphaeropsidales, Melanconiales and about one-fourth of the Moniliales (34), have slime spores (28) spread by contact—with an animal, for example—or by water. Many different animals disseminate spores in sundry ways.

Insects have many relations with fungi, ably summarized by Leach (27). They may gather spores, particularly slime-spores, accidentally; often they are attracted to a spore-mass by odor (*e.g.*, Phallales, decomposing vegetation), taste (*e.g.*, a sweet secretion accompanying the conidia of ergot and the pycniospores of rusts), sight (brightly colored spore masses), or by appeals to two or three of these or comparable senses. Spores are then carried away on or in the insect's body.

Birds, particularly migratory birds, may carry spores long distances.

Man and his transport agencies have disseminated many fungi over the world. We can frequently trace such dispersal of parasitic fungi, commonly spread with seeds or other parts of plants,

but it is seldom possible to decide whether a saprophyte is native or introduced.

Water. Rivulets, streams and spattering rain spread fungi. Zoospores of Myxomycetes and Phycomycetes develop and propel themselves in water. Floating leaves, stems or fruits may transport fungi in fresh or even in sea water. Many spores will float. Hygroscopic movements may aid spore discharge.

Several agencies and factors may interact to determine the effective distribution of an organism. A living fungus spore may travel far, but it must land on the proper host or organic substratum, then develop more spores, despite all vicissitudes. Even then the species may not be able to persist. We know so little of the distribution of most fungi that we can seldom say when, whence, often not even whether, a fungus has arrived in any country.

EFFECT OF CLIMATE ON DISTRIBUTION

Climate is of primary importance in the distribution of vascular plants, the host or substratum in the distribution of fungi. In other words, climate affects fungi more indirectly, or at a stage once removed. Phanerogams cultivated over wide areas, *e.g.*, corn, tomatoes, potatoes, have numerous parasites that attack them anywhere. Nevertheless, climate has a controlling effect on the distribution of many fungi, including various parasites of crop plants.

We are not surprised to find certain species of *Mucor* or *Botrytis* everywhere, of *Peziza* or *Lycoperdon* almost anywhere, of *Puccinia* or *Cercospora* wherever a host thrives. But the range of a fungus is often quite different from that of its hosts or substrata; climatic or other factors must then be involved in limiting its distribution.

Dry regions are characterized by xerophilous Gasteromycetes, Agaricaceae and other fungi, many of which are restricted further to warmer dry localities. *Phymatotrichum omnivorum*, the cause of Texas root-rot of cotton, is definitely known only in southwestern North America; it attacks hundreds of hosts but has not followed them northward nor into moist regions. A dry atmosphere of course limits the development of many leaf parasites and others, but may even favor some, such as certain powdery mildews.

Damp temperate regions, such as those of northern Europe and eastern North America, are generally taken to have the "standard" fungus flora, *i.e.*, the one first and best known.

The moist tropics contain many fungi, a considerable proportion of which are found nowhere else. Most of the accompanying phanerogams are of course also limited to the tropics; but with some groups of fungi it is evidently the climate, not the host, that controls distribution. The Meliolaceae, for example, develop abundantly on diverse hosts in warmer regions; they are few and rare even on similar hosts in cooler areas.

A fungus in the tropics does not have to tide itself over a cold winter, though it generally has to be prepared for a dry spell. Erysiphaceae and various rusts in warm areas persist in their conidial stages.

Arctic regions seem to have high proportions of Pyrenomycetes, frequently without conidial stages, and perhaps more plurivorous than those in temperate countries (18).

Bose (10) reported that the phanerogams and a half dozen Polyporaceae of the Lokra Hills, India, are European in character. He rightly considered that the hosts were of primary importance in the distribution of these polypores; but I think we may say that climate also had an effect. He includes *Polyporus sulphureus* and *P. squamosus*, species with a wide host range in the North Temperate Zone, the former being known in Britain on various dicotyledonous and coniferous trees. I do not know how or when these two fungi reached the Lokra Hills (they occur also in Himalayan areas), but, since their potential host range is so wide, it would seem that climate helps to keep them there.

Temperature, precipitation, humidity, length of season and other climatic factors affect the distribution of fungi. It is not always easy to evaluate these factors. *Claviceps purpurea*, the cause of ergot, would seem to be limited by temperature to temperate regions; but it is possible that *Cerebella*, a fungus which grows on conidial (*Sphacelia*) stages in warm countries, may be more important than climate.

AGE AND AREA

Every student of distribution is in debt to Willis (36, 37) for his extensive compilations and analyses. A few data in this and subsequent sections may be interpreted as having a bearing on his hypothesis of Age and Area, *i.e.*, that (with qualifications) the area occupied by a group of allied species depends chiefly upon or varies directly with their ages.

The exact geographic distribution is known for few of the 3,600 genera and 37,000 species of fungi accepted as "good" (1, 7). Nearly half (47.2%) of the genera of fungi are at present monotypic, as compared with 38.6% of the genera of vascular plants. Fungi show the "hollow curve" stressed by Willis, *i.e.*, the decreasing number of genera having 1, 2, 3 . . . species. The figures for representative groups of fungi are given in Table I.

TABLE I
NUMBERS OF GENERA ARRANGED ACCORDING TO NUMBERS OF SPECIES

		Numbers of genera of			
		Uredinales	Agaricales	Mucorales	Hyphomycetes of Britain
		(1)	(1)	(1)	(34)
Genera having					
1 species	45	25	15	108
2 "	18	14	8	36
3 "	8	2	3	32
4 "	6	2	4	12
5-9 "	16	22	6	29
10-19 "	11	16	3	15
20-29 "	5	11	2	8
30-49 "	3	19	1	3
50-99 "	6	20	..	2
100-199 "	1	22
200-1000 "	3	7
Over 1000 "	1

Willis finds, in general, that the more species per genus, the greater the area covered; the older the genus or species, the greater its area. An analysis for Uredinales is given below.

Rhizophagus is one of the very few fungi that can be traced through the geologic ages. It is now widely distributed on underground parts of Hepaticae, ferns and flowering plants; a fungus of similar morphology is found on fossil remains back to those of the Psilophytales, the earliest land plants (12). Despite its age and wide distribution, *Rhizophagus* is credited with only three species.

Myxomycetes are generally considered to be amongst the more primitive organisms. They are also very widely distributed, though there are relatively few species. Not much is known of the distribution of most other older groups, such as the Chytridiales.

Some fungi are "being evolved" today, *i.e.*, attaining characters which lead mycologists to name them as new species or genera.

Mutations are frequently recognized in the laboratory, sometimes in the field. Parasitic fungi are likely to adapt themselves, with or without morphologic changes, to a new phanerogam as, or soon after, it appears; saprophytic fungi, on the other hand, are unlikely to require any adaptation to the remains of a new plant.

DISTRIBUTION OF GENERA OF FUNGI

An analysis of reported distribution of genera of all fungi (accepted in (1)) is given in Tables II to IV. It will be understood: (a) that many generic names have little or no real basis, or are recognised by one mycologist and not by another; (b) that the actual distribution of most genera is greater than now known; (c) that the figures frequently represent numbers and activity of mycologists rather than distribution: for example, 740 genera are "endemic" in Europe, only 194 in North America. This is approximately the ratio of European and North American taxonomists (237 and 50) listed in our dictionary (1), with allowance for the fact that European mycologists have described numerous North American genera. It is true that only a half dozen mycologists have devoted themselves exclusively to naming the fungi of South America where 280 endemic genera are reported; but the activity of Spegazzini and of taxonomists outside South America has made up for any shortage of workers. Africa, with only 100 genera supposedly endemic, is still rather dark—or conservative—mycologically.

Despite the uncertainties, it seems clear that the distribution of fungi differs from that of phanerogams. Considerable collecting in Ceylon and in Hawaii—*islands noted for high percentages of endemics*—has shown only 12 and 15 genera, respectively, of fungi not yet known elsewhere. The considerable number, 72 genera, known only in the Philippines is likely to be revised in time, though one would expect endemic fungi there. In areas such as Canada, with only a half dozen genera of fungi not known elsewhere, and Manitoba with no endemic genus of fungi or of vascular plants, the distribution of fungi is like that of phanerogams.

Table III gives an approximate summary of Table II. It indicates more clearly that many genera and therefore species of fungi are widely distributed.

TABLE II
RECORDED DISTRIBUTION OF "GOOD" GENERA UP TO 1940

	All fungi	Rusts	Lycoperdales	Phallales
Cosmopolitan	160	5	1	..
Widespread, including Temperate Zone	550	10	4	8
Widespread, tropical & subtropical regions	185	11	1	5
North & South Temperate Zones	183	..	4	..
North Temperate Zone	183	19
Europe	740	1	1	..
Europe & North America ..	217
Europe & North & South America	55
Europe & South America ..	25
Europe & Africa	17	2
Europe & Asia	18	1
Europe & Australasia	7
North America, north of Central America	194	8	1	..
Central America	65	4
West Indies	55	4	..	1
South America	280	17	..	5
North & South America, including temperate regions .	53	3
North & South America, tropical regions	27	11
North & South America & Africa	25	1	3	1
North & South America & Australia-New Zealand ...	13
North & South America & temperate Asia	22	2
North & South America & East Indies	27
Africa	100	4	1	3
Africa & Asia	27	3
Africa & Australia	2
Asia, temperate	44	2
Asia, tropical and East Indies	191 ¹	5	1	2
Asia, Japan	36	4
Asia, India	18	3	1	..
Asia, China & vicinity	6	1
Asia & Australasia	7	1
Australia &/or New Zealand	37	1	3	1
Hawaii	15
Total	3,584	123	21	26

¹ 72 recorded only in the Philippines, 12 only in Ceylon.

Another calculation gives the figures shown in Table IV, where the column for "all fungi" is influenced by the greater study given to fungi in temperate regions.

TABLE III
DISTRIBUTION OF NUMBERS AND PERCENTAGES OF GENERA

Area	All fungi		Uredinales		Phallales & Lycoperdales	
4-6 continents	700,	19.4%	15,	12.2%	16,	34%
About 3 continents	600,	16.7	31,	25.2	6,	13
About 2 continents	500,	13.9	23,	18.7	5,	11
1 continent to 1 locality ..	1,800,	50.0	54,	43.9	20,	42
Total	3,600,	100%	123,	100%	47,	100%

Table V gives an idea of the number of genera of fungi recorded from each of the continents. For "all fungi" the figures have been

TABLE IV
GENERA IN TEMPERATE AND TROPICAL REGIONS

	All fungi		Uredinales		Lycoperdales	Phallales
Temperate	1,600,	44%	35,	28.5%	11	3
Tropical and sub-tropical	1,000,	28	56,	45.5	3	15
Temperate and tropical	1,000,	28	32,	26.0	7	8

reduced in an effort to discount dubious names and useless "segregates".

TABLE V
APPROXIMATE NUMBERS OF GOOD GENERA REPORTED FROM EACH CONTINENT

	All fungi	Uredinales	Phallales and Lycoperdales
Europe	1,800	45	18
North America	1,700	70	22
South America	1,100	52	24
Asia	1,100	56	22
Africa	800	30	22
Australia-New Zealand	600	15	22

The figures for all fungi in Tables II-V leave one rather confused. We turn, therefore, to three orders with better known distribution: the Uredinales, all obligate parasites, and the Phallales and Lycoperdales, all saprophytes.

DISTRIBUTION OF UREDINALES

Distribution of rusts is dependent on that of their hosts—or even, for heteroecious rusts, on that of two dissimilar kinds of hosts. Nevertheless, since a species of rust is usually able to attack more than one species of host, the average range of a rust may be greater than that of a host.

It is somewhat surprising to note that only two species of rust, and only two collections of each, have been found on the Krakatau islands; for we know that many rusts, notoriously *Puccinia graminis*, travel far by means of air-borne urediospores.

About 60% of the rusts of North America are endemic; at least 40 species (4%) have been introduced by man (2). Temperate parts of that continent have the largest number of rusts; boreal parts have the fewest but show a higher percentage of Melampsoraceae and of microcyclic species.

An analysis based on Table I shows that genera with more than 200 species (*Puccinia*, *Uromyces*, *Aecidium*, *Uredo*) are all cosmopolitan, though the genus (*Ravenelia sensu lat.*) next in size is almost confined to Leguminosae and to warmer areas. The 45 monotypic genera are mostly restricted in distribution: North Temperate Zone, 2; Europe, 1; Europe and Africa, 2; Africa, 4; Java, 1; Philippines, 2; Japan, 3; India, 2; North America (Mexico north), 7; Central America, 4; West Indies, 4; South America, 12; Tropical America (North and South), 1. All this is in harmony with "Age and Area". One rather striking fact is that 28 (62%) of these monotypic genera are known only in the Americas, mostly in tropical or sub-tropical areas. This perhaps to some extent reflects the rather greater study of rusts in the western hemisphere than in tropical Africa or Asia; but it suggests that warmer American regions are or have been a main center of rust development. Genera with two and three species, though naturally rather more widely distributed than monotypic genera, are also to a considerable extent American. *Hemileia*, however, is predominantly African, and about half its species attack Rubiaceae.

Cummins (16) has made a thorough study of *Prospodium*. All 50 species, except one doubtful form of *Uredo* from Ceylon, known only from the type collection showing no telia, have been found in warmer American regions. Most of them are on Bignoniaceae, 22 genera of which are recorded as hosts; nine species are on Verben-

ceae—one on *Lantana*, eight on *Lippia*. No other host family is known. The Bignoniaceae occur particularly in South America; the Verbenaceae, though also mostly limited to warmer countries, are not predominantly American. I conclude that hosts primarily, then climate, limit the distribution of *Prospodium*; that the genus and species obviously evolved in warmer America; that for some reason, possibly lack of time and opportunity, little or no spread to other countries and other related hosts has taken place.

It is noteworthy that only about 30 genera of Uredinales (four endemic) were recorded from Africa up to 1940 (Tables II–V); that only 15 were known in Australia and New Zealand, only one of which is endemic despite the high proportion of endemic higher plants which exist there as potential or actual hosts. New Zealand (17) has 53% of endemic species of rusts, rather less than the North American percentage, but, as might be expected, it has nearly as many introduced species (33 in New Zealand, 40 in North America).

I am inclined to look with suspicion upon a fungus which remains “known only from the type locality”. There are some 35 rusts, exclusive of those in form genera, in this class in the United States and Canada. Uredinologists will doubtless seek explanations for such apparent rarity; perhaps some of these species are already eligible for a group of fungi hitherto unknown, at least to me, namely, those that have definitely disappeared from the earth since man described them.

It is well known that similar rusts occur in alpine regions in various parts of the world; for example, 22% of the rusts in alpine regions in Japan are found also in the Swiss Alps (23). I interpret this as primarily a host relationship, though climate is involved, as it is particularly in the shortening of life cycles at high altitudes.

The distribution of many rusts seems perplexing at the moment (e.g., *Trillium* rust (3)), but important facts regarding the distribution and evolution of parasitic fungi have come and will come from studies on Uredinales (26). Many of them are widespread, more than a third of the genera being known from three or more continents (Table III).

Crowell (15), in a comprehensive study of the distribution of *Gymnosporangium*, concludes that various factors limit many species to ranges less than that of their hosts, and considers this quite

the reverse of my general finding (5). It still must be remembered, however, that most species of *Gymnosporangium* have more than one aecial or telial host. Take *G. juvenescens*, for example; its distribution seems to cover only about half that of its hosts—but Arthur records it on eleven aecial and three telial hosts. My thesis was that an average rust covers more range than an average host, but not necessarily more than a dozen of its hosts.

DISTRIBUTION OF GASTEROMYCETES

The conspicuous or even bizarre nature of many Gasteromycetes has resulted in exceptional knowledge of their distribution. Australia-New Zealand stand out by having about as many genera and as many endemic species as are to be found in any larger continental area. Primitive Gasteromycetes are found particularly in regions bordering the Pacific, indicating that these regions have been centers for development of the class (21). An analysis of genera of Phallales and Lycoperdales having one, two and three species suggests the same conclusion: about half such genera in each of the two orders are known near the Pacific. It is perhaps even more noteworthy that of eleven monotypic genera of Phallales, ten are restricted to warm and relatively small areas in America, Asia or Africa, the eleventh to New Zealand. Table IV shows that the genera of Phallales are predominantly tropical, of Lycoperdales temperate. Nearly half the genera in these orders are still known only within a single continent, in spite of the fact that man has introduced several Phallales to distant countries: *Lysurus* and *Aseroe* to Britain, for example; probably *Colus ?javanicus* to the United States (31). One may conclude that many Phallales had a rather limited distribution before man began to travel; in other words, that they are influenced by climate and probably by having had less time than many other fungi in which to travel by their own devices.

Other Gasteromycetes worthy of mention are *Queletia* with one species known from three collections in France, one in Britain, and a couple in successive years in one spot in Pennsylvania; and *Podaxis* with one species in dry regions within about 40° north and south of the equator (29). Incidentally, the dependence of knowledge of distribution upon that of taxonomy is well illustrated with *Podaxis*; before Miss Morse's study one might have tabulated a

couple of dozen "species" of the genus, many of them recorded from only one locality.

Few saprophytic fungi in other groups can be so definitely recognized as having been introduced to an area. Many Gastromycetes are widespread, but it is not surprising that some of these specialized "highest" fungi should have limited natural ranges. Once more we see that climate is important, even to obligate saprophytes.

DISTRIBUTION OF OTHER GROUPS

Myxomycetes are amongst the best known, most widely distributed and presumably oldest of fungal groups. We have noted that 28 species, 7% of all, are reported to have reached the Krakatau islands.

Little is known of the distribution of most of the simple, often aquatic, Phycomycetes; but some Peronosporales and Zygomycetes are cosmopolitan. During the last few years, Drechsler (22) has found a whole new family, the Zoopagaceae, belonging to the Entomophthorales; the species are probably widely distributed, but at present most of them are reported from only one or two American collections.

Ascomycetes contain many widespread fungi and many with unknown distribution. What can a student of plant geography do with a genus such as *Dipodascus*? It has two species, one described 50 years ago from Ecuador, and since then recognised twice, from Sweden and Colombia; the second species has but one record, from Canada (4). The Laboulbeniales seem to develop most abundantly in the tropics, though 150 species are said to be palaearctic, with more than 50 recorded from Poland (32).

The Hymenomycetes of the north temperate area are similar everywhere; some species, such as *Armillaria mellea*, extend over much of the world. In most groups, however, the species in tropical or south temperate regions are to a considerable extent distinct from those in the north temperate. It should be noted, however, that coprophilous agarics and other coprophilous fungi are almost as ubiquitous as their substratum; that the genera of Hymenomycetes in the south temperate (13) are about the same as those in the north, and that even in the torrid zone the majority of genera of Hymenomycetes are those of temperate regions.

Overholts, in an important contribution (30) to the knowledge of geographical distribution, finds that 43% of the American pileate

Polyporaceae are known in Europe or other parts of the eastern hemisphere, but that many species are limited by climatic factors. He has been able to map a number of species, and to predict extensions to their known distribution in the United States and Canada. When accurate maps such as his become available for other groups of fungi, we can start to make more definite generalizations on distribution.

Many Fungi Imperfecti, particularly Hyphomycetes of the "mould" group such as those in the soil, are to be found anywhere.

DISTRIBUTION OF FUNGI PARASITIC ON CROP PLANTS

It is scarcely necessary to discuss in detail the distribution of crop diseases. Maps are now being published (24) showing the range of many. Man has been very active in assisting nature; for example, asparagus rust was enabled to establish itself from New Jersey to California in five years.

A few pathogens are worthy of mention because they seem to illustrate principles. *Puccinia Antirrhini*, the rust of snapdragons, is native to a few wild Scrophulariaceae in the mountains of California. Soon after *Antirrhinum majus* was introduced there it was attacked and proved to be a very congenial host; the rust spread on snapdragons throughout the United States and Canada, and now occurs over much of Europe and in Egypt, Palestine and South Africa.

The original host of *Synchytrium endobioticum*, the cause of wart disease of potatoes, is not known. Potatoes were in general culture in Europe for about 150 years (35) before the fungus was described on them in 1896. It then spread over northern Europe and reached Newfoundland and South Africa, but its late start permitted prevention of its spread over North America.

Spongospora subterranea, another parasite of the potato, has been known for a century. It has spread far. Considerable alarm was felt in North America after it was first found in Canada in 1913, but subsequent experience demonstrated that climate almost limits it to cool regions such as those near the United States-Canada boundary in the east and west. The fungus seems to be unimportant, except perhaps at high altitudes, in Asia, Africa and South America. Possibly a consideration of the effect of climate on this and other pathogenic fungi would permit some modification of the elaborate quarantine and inspection regulations imposed by most

countries. Unfortunately, we do not yet know how much faith to place in climate. Can we be sure that *Claviceps purpurea* will not develop in the tropics, and only in Algeria and Morocco in Africa?

Cronartium ribicola has now spread over most north temperate regions where its hosts grow, *Ribes* and five-needle pines in association. The same is true of many another parasite of economic plants. In other words, the host is of primary importance in dissemination of parasites. It would be hard to stop nature and man in their efforts to spread pathogens, were it not that climatic and other factors are also important, and may hinder as well as favor spread.

RESTATEMENT

Comprehensive facts and principles regarding geographical distribution are scarcely possible until many more fungi can be mapped accurately. Taxonomy is badly in need of synthesis, so that mycologists everywhere can speak in the same terms. Much progress has, however, been made during the decade since I previously attempted to summarize the subject.

The morphological and physiological devices and processes enabling fungi to present their spores for dispersal are now seen much more clearly. Spores have been tracked through the upper air, across continents, and to new Krakatau. A more definite picture of the mycological flora of the tropics and south temperate is developing.

We conclude (7), after going over the fungi genus by genus, that less than 40,000 good species are known today, and many of these are "host-species" based on host plus morphology. Some 60% of all species are saprophytes. Perhaps 100,000 or even more species may be present on earth. There are about 200,000 known species of phanerogams, and I suppose about 100,000 still unknown, unless taxonomists turn more conservative and reduce their numbers.

Climate has a controlling effect on the distribution of many fungi. There are other factors, such as plant succession, amount of disturbance of the environment, light and length of season, the effects of which are difficult to evaluate. Study of the distribution of monotypic genera and of important parasites or other species which can be mapped in detail, with application of the principles of statistics, philosophy, and "Age and Area", seem likely to prove illuminating.

I now venture this revised summary to replace the one I made ten years ago:

1. Species of phanerogams acceptable today outnumber such species of fungi by about five to one. There are perhaps three times as many phanerogams as fungi on earth.

2. Nevertheless, species of fungi can be expected to outnumber species of phanerogams in any particular state or country. In other words, the average range of a fungus is the greater.

3. The smaller the area surveyed, the more the fungi predominate.

4. Though certain fungi were doubtless amongst the most primitive inhabitants of the earth, they have come to depend largely on phanerogams and their remains.

5. Saprophytic fungi generally have wider distribution than parasitic.

6. Distribution of hosts and substrata primarily controls distribution of fungi. A parasite is commonly able to attack more than one species of host, so that its range can be greater than that of any one of its hosts.

7. Climate has an important influence on many fungi. Even obligate saprophytes may be limited to certain climatic areas.

8. Other factors, including the nature and density of phanerogams, light, and particularly the activities of man, influence the distribution of many fungi.

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Proposed Future Contents of THE BOTANICAL REVIEW

Articles received and awaiting publication

Plant Life and the Law of Man. IV. Barberry, Currant and Gooseberry, and Cedar Control ...	E. H. FULLING <i>The Botanical Review</i>
Effects of Fire on Vegetation of the Southeastern United States	KENNETH H. GARREN <i>Georgia Agricultural Experiment Station</i>
The Cuticle in Angiosperms	J. H. PRIESTLEY <i>University of Leeds</i>
Lichens—Their Biological and Economic Signifi- cance	G. A. PEREZ-LLANO <i>Harvard University</i>
Root-rots of Some Non-cereal Crops	G. H. BERKELEY <i>Dominion Laboratory of Plant Pathology</i>
Recent Developments in the Classification of Bac- terial Plant Pathogens	CHARLOTTE ELLIOTT <i>Bureau of Plant Industry</i>
Phytogeography of Patagonia	A. A. BEETLE <i>University of California</i>
The Classification of Inflorescences	H. W. RICKETT <i>New York Botanical Garden</i>

Articles arranged for most recently

Cytogenetics of Nicotiana	T. H. GOODSPEED <i>University of California</i>
Tundra Vegetation	R. F. GRIGGS
Cytology and Genetics in Relation to Taxonomy ..	C. L. HUSKINS <i>University of Toronto</i>

Articles in course of preparation

The Cytology of Fertilization in Angiosperms	L. E. ANDERSON <i>Duke University</i>
Development of the Madre-Tertiary Flora	D. I. AXELROD <i>University of California</i>
Relation of Wood Anatomy to Taxonomy	I. W. BAILEY <i>Harvard University</i>
Anthocyanin Pigments	F. BLANK <i>Switzerland</i>
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